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(54) Title: TRP8, TRP9 AND TRP10, NOVEL MARKERS FOR CANCER

(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

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**Trp8, Trp9 and Trp10, novel markers for cancer**FIELD OF THE INVENTION

The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10

BACKGROUND OF THE TECHNOLOGY

Prostate cancer is one of the most common diseases of older men world wide. Diagnosis and monitoring of prostate cancer is difficult because of the heterogeneity of the disease. For diagnosis different grades of malignancy can be distinguished according to the Gleason-Score Diagnosis. For this diagnosis a prostate tissue sample is taken from the patient by biopsy and the morphology of the tissue is investigated. However, this approach only yields subjective results depending on the experience of the pathologist. For confirmation of these results and for obtaining an early diagnosis an additional diagnostic method can be applied which is based on the detection of a prostate specific antigen (PSA). PSA is assayed in serum samples, blood samples etc. using an anti-PSA-antibody. However, since in principle PSA is also expressed in normal prostate tissue there is a requirement for the definition of a threshold value (about 4 ng/ml PSA) in order to be able to distinguish between normal and malign prostate tissue. Unfortunately, this diagnostic method is quite insensitive and often yields false-positive results. Moreover, by using this diagnostic method any conclusions as regards the grade of malignancy, the progression of the tumor and its potential for metastasizing cannot be drawn. Thus, the use of molecular markers would be helpful to distinguish benign from malign tissue and for grading and staging prostate carcinoma, particularly for patients with metastasizing prostate cancer having a very bad prognosis.

The above discussed limitations and failings of the prior art to provide meaningful specific markers which correlate with the presence of prostate tumors, in particular metastasizing tumors, has created a need for markers which can be used diagnostically, prognostically and therapeutically over the course of this disease. The present invention fulfils such a need by the provision of Trp8, Trp9 and Trp10 and the genes encoding Trp8, Trp9 and Trp10: The genes encoding Trp8 and Trp10 are expressed in prostate carcinoma and prostatic metastasis, but

not in normal prostate, benign hyperplasia (BHP) and intraepithelial prostatic neoplasia (PIN). Furthermore, expression of Trp10 transcripts is detectable in carcinoma but not in healthy tissue of the lung, the prostate, the placenta and in melanoma.

### SUMMARY OF THE INVENTION

The present invention is based on the isolation of genes encoding novel markers associated with a cancer, Trp8, Trp9 and Trp10. The new calcium channel proteins Trp8, Trp9 and Trp10 are members of the trp (transient receptor potential) - family, isolated from human placenta (Trp8a and Trp8b) and human prostate (Trp9, Trp10a and Trp10b). Trp proteins belong to a steadily growing family of  $\text{Ca}^{2+}$  selective and non selective ion channels. In the recent years seven Trp proteins (trp1 - trp7) have been identified and suggested to be involved in cation entry, receptor operated calcium entry and pheromone sensory signaling. Structurally related to the trp proteins are the vanilloid receptor (VR1) and the vanilloid like receptor (VRL-1) both involved in nociception triggered by heat. Furthermore, two calcium permeable channels were identified in rat small intestine (CaT1) and rabbit kidney (ECaC). These distantly related channels are suggested to be involved in the uptake of calcium ions from the lumen of the small intestine (CaT1) or in the reuptake of calcium ions in the distal tubule of the kidney (ECaC). Common features of the Trp and related channels are a proposed structure comprising six transmembrane domains including several conserved amino acid motifs. In the present invention the cloning and expression of a CaT1 like calcium channel (Trp8) from human placenta as well as Trp9 and Trp10 (two variants, Trp10a and Trp10b) is described. Two polymorphic variants of the Trp8 cDNA were isolated from placenta (Trp8a and Trp8b). Transient expression of the Trp8b cDNA in HEK (human embryonic kidney) cells results in cytosolic calcium overload implicating that the Trp8 channel is constitutive open in the expression system. Trp8 induces highly calcium selective inward currents in HEK cells. The C-terminus of the Trp8 protein binds calmodulin in a calcium dependent manner. The Trp9 channel is expressed in trophoblasts and syncytiotrophoblasts of placenta and in pancreatic acinar cells. Furthermore, the Trp8 channel is expressed in prostatic carcinoma and prostatic metastases, but not in normal tissue of the prostate. No expression of Trp8 transcripts is detectable in benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN). Therefore, the Trp8 channel is exclusively expressed in malign prostatic tissues and serves as molecular marker for prostate cancer. From the experimental results it is also apparent that the

modulation of Trp8 and/or Trp10, e.g. the inhibition of expression or activity, is of therapeutic interest, e.g. for the prevention of tumor progression.

The present invention, thus, provides a Trp8, Trp9 and Trp10 protein, respectively, as well as nucleic acid molecule encoding the protein and, moreover, an antisense RNA, a ribozyme and an inhibitor, which allow to inhibit the expression or the activity of Trp8, Trp9 and/or Trp10.

In one embodiment, the present invention provides a diagnostic method for detecting a prostate cancer or endometrial cancer (cancer of the uterus) associated with Trp8 or Trp10 in a tissue of a subject, comprising contacting a sample containing Trp8 and/or Trp10 encoding mRNA with a reagent which detects Trp8 and/or Trp10 or the corresponding mRNA.

In a further embodiment, the present invention provides a diagnostic method for detecting a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense transcripts or Trp10a and/or Trp10b related antisense transcripts.

In another embodiment, the present invention provides a method of treating a prostate tumor, carcinoma of the lung, carcinoma of the placenta (chorion carcinoma) or melanoma associated with Trp8 and/or Trp10, comprising administering to a subject with such an disorder a therapeutically effect amount of a reagent which modulates, e.g. inhibits, expression of Trp8 and/or Trp10 or the activity of the protein, e.g. the above described compounds.

Finally, the present invention provides a method of gene therapy comprising introducing into cells of a subject an expression vector comprising a nucleotide sequence encoding the above mentioned antisense RNA or ribozyme, in operable linkage with a promoter.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1: A, phylogenetic relationship of trp and related proteins. B, hydropathy plot of the Trp8 protein sequence according to Kyte and Doolittle. C, alignment of Trp8a/b to the epithelial calcium channels ECaC (from rabbit) and Vr1 (from rat). Putative transmembrane domains are underlined.

**Figure 2:** A, polymorphism of the Trp8 gene. The polymorphic variants Trp8a and Trp8b differ in five base pairs resulting in three amino acid exchanges in the derived protein sequences. Specific primers were derived from the Trp8 gene as indicated by arrows. B, the Trp8a and Trp8b genes are distinguishable by a single restriction site. Genomic fragments of the Trp8 gene can be amplified using specific primers (shown in A). The genomic fragment of the Trp8b gene contains an additional site of the restriction enzyme BSP1286I (B). C, the Trp8 gene is located on chromosome 7. D, genotyping of eleven human subjects. A 458 bp genomic fragment of the Trp8 gene was amplified using specific primers (shown in A) and restricted with BSP1286I. The resulting fragments were analyzed by PAGE electrophoresis.

**Figure 3:** The Trp8b protein is a calcium selective ion channel. A, representative trace of a pdiTrp8b transfected HEK 293 cell. Trp8b mediated currents are activated by voltage ramps (-100 mV - +100 mV) of 100 msec at -40 mV or +70 mV holding potential. 1, Trp8b currents in the presence at 2mm  $[Ca^{2+}]_o$ ; 2, effect of solution switch alone 3, switch to nominal zero calcium solution. B, Trp8b currents in the presence of zero divalent cations. C, current voltage relationship of the currents shown in A. Inset, leak subtracted current. D, current voltage relationship of the current shown in B. E, statistics of representative experiments. Black: Trp8 transfected cells, gray: control cells. Columns from left to right: Trp8 currents at - 40 mV ( $n=12$ ) and + 70 mV holding potential ( $n=12$ ). Trp8 currents in standard bath solution including 120 mM NMDG without sodium ( $n=7$ ) and with nominal zero calcium ions ( $n=8$ ) or in the presence of 1mM EGTA with zero divalent cations ( $n=6$ ). F, representative changes in  $[Ca^{2+}]_i$  in Trp8b transfected HEK cells (gray) and controls (black) in the presence or absence of 1mM  $[Ca^{2+}]_o$ . Inset, relative increase of cytosolic calcium concentration of Trp8b transfected HEK cells, before and after readdition of 1 mM  $[Ca^{2+}]_o$  in comparison to control cells.

**Figure 4:** The C-terminal region of the Trp8 protein binds calmodulin. A, N- and C-terminal fragments of the Trp8 protein used for calmodulin binding studies. B, the Trp8 protein and a truncated Trp8 protein which was in vitro translated after MunI cut of the cDNA, which lacks the C-terminal 32 amino acid residues, were in vitro translated in the presence of  $^{35}S$ -methionine and incubated with calmodulin coupled agarose beads in the presence of 1 mM  $Ca^{2+}$  or 2 mM EGTA. C, calmodulin binding to N- and C-terminal fragments of the Trp8protein in the presence of  $Ca^{2+}$  (1 mM) or EGTA (2 mM)

Figure 5: Expression pattern of the Trp8 cDNA. A, Northern blots (left panels, Clontech, Palo Alto) were hybridized using a 348 bp NcoI/BamHT fragment of the Trp9 cDNA. The probe hybridizes to mRNA species isolated from the commercial blot, but not to mRNA species isolated from benign prostate hyperplasia (right panel, mRNA isolated from 20 human subjects with benign prostate hyperplasia). B,C, *in situ* hybridization with biotinylated Trp8 specific oligonucleotides on slides of human tissues. Left column antisense probes, right column sense probes. D, antisense probes.

Figure 6: Differential expression of Trp8 cDNA in human prostate. A - F, *in situ* hybridization with prostatic tissues. A, normal prostate, B, primary carcinoma, C, benign hyperplasia, D, rezidive carcinoma, E, prostatic intraepithelial neoplasia, F, lymphnode metastasis of the prostate.

Figure 7: Trp8a cDNA sequence and derived amino acid sequence

Figure 8: A, Trp8b cDNA sequence and derived amino acid sequence

B, cDNA sequence of splice variant 1 (12B1)

C, cDNA sequence of splice variant 2 (17-3)

D, cDNA sequence of splice variant 3 (23A3)

E, cDNA sequence of splice variant 4 (23C3)

Figure 9: A, Trp9 cDNA sequence and derived amino acid sequence B, cDNA sequence of splice variant 15 and derived amino acid sequence.

Figure 10: A, cDNA sequence of Trp10a and derived amino acid sequence, B, cDNA fragment of Trp10a and derived amino acid sequence.

Figure 11: cDNA sequence of Trp10b and derived amino acid sequence.

Figure 12: Expression of Trp8 mRNA in human endometrial cancer or cancer of the uterus. A - D, *in situ* hybridization with slides of endometrial cancer hybridized with Trp8 antisense (left column) or sense probes as controls (right column). E - F, Trp8 antisense probes hybridized to slides of normal endometrium. It can be clearly seen no hybridization occurs with normal endometrial tissue.

**Figure 13: Expression of human Trp9 and Trp10 genes**

Northern blots were hybridized using Trp9 (upper panel) or Trp10 (lower panel) specific probes. Expression of the Trp9 cDNA is detectable in many tissues including human prostate and colon as well as in benign prostatic hyperplasia. Expression of Trp10 cDNA is detectable in human prostate of a commercial northern blot (Clontech, right side). This Northern blot contains prostatic tissue collected from 15 human subjects in the range of 14 - 60 years of age. No expression of Trp10 cDNA was detectable in benign prostatic hyperplasia (left side).

**Figure 14: Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and metastasis of a melanoma.** In situ hybridizations of slides hybridized with Trp10-antisense (A-E, K-N) and Trp10 related sense probes (F-J, P-R). It can clearly be seen that both probes detect the same cancer cells indicating that these cancer cells express Trp10 transcripts as well as Trp10-antisense transcripts. S, no Trp10 expression is detectable in benign hyperplasia of the prostate (BPH). O and T, show expression of Trp10 transcripts (O) and Trp10-antisense transcripts (T) in a metastasis of a melanoma in human lung. Melanoma cancer cells express both Trp10 transcripts and Trp10-antisense transcripts.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

- (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11;
- (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10, or 11;
- (c) a nucleic acid molecule included in DSMZ Deposit no. DSM 13579 (deposit date: 28 June 2000), DSM 13580 (deposit date: 28 June 2000), DSM 13584 (deposit date: 5 July 2000), DSM 13581 (deposit date: 28 June 2000) or DSM ....(deposit date:....);
- (d) a nucleic acid molecule with hybridizes to a nucleic acid molecule specified in (a) to (c)

- (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
- (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

As used herein, a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b is understood to be a protein having at least one of the activities as illustrated in the Examples, below.

As used herein, the term „isolated nucleic acid molecule,“ includes nucleic acid molecules substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated.

In a first embodiment, the invention provides an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11. The present invention also provides a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11.

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The present invention provides not only the generated nucleotide sequence identified in Figure 7, 8A, 9, 10 or 11, respectively and the predicted translated amino acid sequence, respectively, but also plasmid DNA containing a Trp8a cDNA deposited with the DSMZ, under DSM 13579, a Trp8b cDNA deposited with the DSMZ, under DSM 13580, a Trp9 cDNA deposited with the DSMZ, under DSM 13584, a Trp10a cDNA deposited with the DSMZ, under DSM 13581, and a Trp10b cDNA deposited with the DSMZ, under DSM..., respectively. The nucleotide sequence of each deposited Trp-clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by each deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited Trp-encoding DNA, collecting the protein, and determining its sequence.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. It is understood that all

nucleic acid molecules encoding all or a portion of Trp8a, Trp8b, Trp9, Trp10a or Trp10b are also included, as long as they encode a polypeptide with biological activity. The nucleic acid molecules of the invention can be isolated from natural sources or can be synthesized according to known methods.

The present invention also provides nucleic acid molecules which hybridize to the above nucleic acid molecules. As used herein, the term „hybridize“, has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual 2<sup>nd</sup> edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Also contemplated are nucleic acid molecules that hybridize to the Trp nucleic acid molecules at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from human cell lines or tissues. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., *supra*). As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic

fragments that were produced by means of conventional synthetic methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The nucleic acid molecules of the present invention also include molecules with sequences that are degenerate as a result of the genetic code.

In a further embodiment, the present invention provides nucleic acid molecules which comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. „Fragments,“ are understood to be parts of the nucleic acid molecules that are long enough to encode one of the described proteins. These fragments comprise nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules of the invention. These nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assay and/or kit described below and, preferably, are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can also be used, for example, as primers for a PCR reaction. Examples of particular useful probes (primers) are shown in Tables 1 and 2.

Table 1

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

1. ) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTTGAGGAT 3'

Tabelle 2

Trp10 probes used for the in situ hybridizations shown in Figure 14:

## Probes (antisense)

- 1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (Figure 14 A, 14B)
- 2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (Figure 14C, 14D, 14N, 14S, 14O)
- 3.) 5' ATCTTCCAGTTCTGGTGTCTCGG 3' (Figure 14E, 14K)
- 4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (Figure 14L, 14M)

## Probes (sense)

- 1.) 5' TCTATTCCGTGAAGCTTGGGTGGAAGC 3' (Figure 14F, 14G)
- 2.) 5' GCCACAGACCAGCATTTCATCGCC 3' (Figure 14H, 14I, 14T)
- 3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (Figure 14J, 14P)
- 4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (Figure 14Q, 14R)

The term „derivative,“ in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and particularly preferred of more than 90%. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in Figure 7, 8A, 9, 10 and 11, respectively, of at least 80%, preferably of 85% and particularly preferred of more than 90%, 97% and 99%. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination. The definition of the derivatives also includes splice variants, e.g. the splice variants shown in Figures 8B to 8E and 9B.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes.

Generally, by means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., *supra*) to introduce different mutations into the nucleic acid molecules of the invention. As a result Trp proteins or Trp related proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. Another possibility is the introduction of single-point mutation at positions where a modification of the amino acid sequence influences, e.g., the ion channel properties or the regulations of the trp-ion channel. By this method muteins can be produced, for example, that possess a modified ion conducting pore, a modified  $K_m$ -value or that are no longer subject to the regulation mechanisms that normally exist in the cell, e.g. with regard to allosteric regulation or covalent modification. Such muteins might also be valuable as therapeutically useful antagonists of Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., *supra*) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, *in vitro* mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as ion channel activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretical mobility, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability, pH optimum, temperature optimum.

The invention furthermore relates to vectors containing the nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors

usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the recombinant vector of the invention that guarantee the transcription and synthesis of an RNA in prokaryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter.

In a further embodiment, the present invention relates to recombinant host cells transiently or stably containing the nucleic acid molecules or vectors of the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention. Preferably, these cells are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The host cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to isolated proteins exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby, e.g., a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced proteins may be carried out by conventional means including preparative chromatography and affinity and immunological separations involving affinity with an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a- or anti-Trp10b-antibody, respectively.

As used herein, the term „isolated protein,“ includes proteins substantially free of other proteins, nucleic acids, lipids, carbohydrates or other materials with which it is naturally associated. Such proteins however not only comprise recombinantly produced proteins but include isolated naturally occurring proteins, synthetically produced proteins, or proteins

produced by a combination of these methods. Means for preparing such proteins are well understood in the art. The Trp proteins are preferably in a substantially purified form. A recombinantly produced version of a human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b protein, including the secreted protein, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67; 31-40 (1988).

In a further preferred embodiment, the present invention relates to an antisense RNA sequence characterised that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to said mRNA, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecules, and a ribozyme characterised in that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to and cleave said mRNA, thus inhibiting the synthesis of the proteins encoded by said nucleic acid molecules. Ribozymes which are composed of a single RNA chain are RNA enzymes, i.e. catalytic RNAs, which can intermolecularly cleave a target RNA, for example the mRNA transcribed from one of the Trp genes. It is now possible to construct ribozymes which are able to cleave the target RNA at a specific site by following the strategies described in the literature. (see, e.g., Tanner et al., in: *Antisense Research and Applications*, CRC Press Inc. (1993), 415-426). The two main requirements for such ribozymes are the catalytic domain and regions which are complementary to the target RNA and which allow them to bind to its substrate, which is a prerequisite for cleavage. Said complementary sequences, i.e., the antisense RNA or ribozyme, are useful for repression of Trp8a-, Trp8b-, Trp9-, Trp10a- and Trp10b-expression, respectively, i.e. in the case of the treatment of a prostate cancer or endometrial cancer (carcinoma of the uterus). Preferably, the antisense RNA and ribozyme of the invention are complementary to the coding region. The person skilled in the art provided with the sequences of the nucleic acid molecules of the present invention will be in a position to produce and utilise the above described antisense RNAs or ribozymes. The region of the antisense RNA and ribozyme, respectively, which shows complementarity to the mRNA transcribed from the nucleic acid molecules of the present invention preferably has a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides.

In still a further embodiment, the present invention relates to inhibitors of Trp8a, Trp8b, Trp9, Trp10a and Trp10b, respectively, which fulfill a similar purpose as the antisense RNAs or

ribozymes mentioned above, i.e. reduction or elimination of biologically active Trp8a, Trp8b, Trp9, Trp10a or Trp10b molecules. Such inhibitors can be, for instance, structural analogues of the corresponding protein that act as antagonists. In addition, such inhibitors comprise molecules identified by the use of the recombinantly produced proteins, e.g. the recombinantly produced protein can be used to screen for and identify inhibitors, for example, by exploiting the capability of potential inhibitors to bind to the protein under appropriate conditions. The inhibitors can, for example, be identified by preparing a test mixture wherein the inhibitor candidate is incubated with Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively, under appropriate conditions that allow Trp8a, Trp8b, Trp9, Trp10a or Trp10b to be in a native conformation. Such an in vitro test system can be established according to methods well known in the art. Inhibitors can be identified, for example, by first screening for either synthetic or naturally occurring molecules that bind to the recombinantly produced Trp protein and then, in a second step, by testing those selected molecules in cellular assays for inhibition of the Trp protein, as reflected by inhibition of at least one of the biological activities as described in the examples, below. Such screening for molecules that bind Trp8a, Trp8b, Trp9, Trp10a or Trp10b could easily be performed on a large scale, e.g. by screening candidate molecules from libraries of synthetic and/or natural molecules. Such an inhibitor is, e.g., a synthetic organic chemical, a natural fermentation product, a substance extracted from a microorganism, plant or animal, or a peptide. Additional examples of inhibitors are specific antibodies, preferably monoclonal antibodies. Moreover, the nucleic sequences of the invention and the encoded proteins can be used to identify further factors involved in tumor development and progression. In this context it should be emphasized that the modulation of the calcium channel of a member of the trp family can result in the stimulation of the immune response of T lymphocytes leading to proliferation of the T lymphocytes. The proteins of the invention can, e.g., be used to identify further (unrelated) proteins which are associated with the tumor using screening methods based on protein/protein interactions, e.g. the two-hybrid-system Fields, S. and Song, O. (1989) *Nature* (340): 245-246.

The present invention also provides a method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.

It has been found that carcinoma cells of placenta (chorion carcinoma), lung and prostate express Trp10 transcripts as well as Trp10 antisense transcripts and transcripts being in part complementary to Trp10 antisense transcripts. Accordingly, the present invention also provides a method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA.

When the target is mRNA (or antisense RNA), the reagent is typically a nucleic acid probe or a primer for PCR. The person skilled in the art is in a position to design suitable nucleic acids probes based on the information as regards the nucleotide sequence of Trp8a, Trp8b, Trp10a or Trp10b as depicted in figure 7, 8a, 10 and 11, respectively, or tables 1 and 2, above. When the target is the protein, the reagent is typically an antibody probe. The term „antibody“, preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing fragments of the proteins of the invention by methods well known to those skilled in the art (see, e.g., Köhler et al., *Nature* 256 (1975), 495). As used herein, the term „antibody“ (Ab) or „monoclonal antibody“ (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and f(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., *J. Nucl. Med.* 24: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies. The target cellular component, i.e. Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, e.g., in biological fluids or tissues, may be detected directly *in situ*, e.g. by *in situ* hybridization (e.g., according to the examples, below) or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern blot analysis, RNase protection, *in situ* methods, e.g. *in situ* hybridization, *in vitro* amplification methods (PCR, LCR, QRNA replicase or RNA-transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362)), immunoassays, Western blot and other detection assays that are known to those skilled in the art.

Products obtained by in vitro amplification can be detected according to established methods, e.g. by separating the products on agarose gels and by subsequent staining with ethidium bromide. Alternatively, the amplified products can be detected by using labeled primers for amplification or labeled dNTPs.

The probes can be detectable labeled, for example, with a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

Expression of Trp8a, Trp8b, Trp10a and Trp10b, respectively, in tissues can be studied with classical immunohistological methods (Jalkanen et al., *J. Cell. Biol.* 101 (1985), 976-985; Jalkanen et al., *J. Cell. Biol.* 105 (1987), 3087-3096; Sobol et al. *Clin. Immunopathol.* 24 (1982), 139-144; Sobol et al., *Cancer* 65 (1985), 2005-2010). Other antibody based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium rhodamine, and biotin. In addition to assaying Trp8a, Trp8b, Trp 10a or Trp10b levels in a biological sample, the protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99}\text{mTc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99}\text{mTc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In

vivo tumor imaging is described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments“. (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

The marker Trp8a and Trp8b is also useful for prognosis, for monitoring the progression of the tumor and the diagnostic evaluation of the degree of malignancy of a prostate tumor (grading and staging), e.g. by using *in situ* hybridization: In a primary carcinoma Trp8 is expressed in about 2 to 10% of carcinoma cells, in a rezidive carcinoma in about 10 to 60% of cells and in metastases in about 60 to 90% of cells.

The present invention also relates to a method for diagnosing endometrial cancer (cancer of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the encoding mRNA and detecting Trp8a and/or Trp8b encoding mRNA. As regards particular embodiments of this method reference is made to the particular embodiments of the method of diagnosing a prostate cancer outlined above.

For evaluating whether the concentration of Trp8a, Trp8b, Trp10a or Trp10b or the concentration of Trp8a, Trp8b, Trp10a or Trp10b encoding mRNA is normal or increased, thus indicative for the presence of a malignant tumor, the measured concentration is compared with the concentration in a normal tissue, preferably by using the ratio of Trp8a:Trp9, Trp8b:Trp9 or Trp10(a or b)/Trp9 for quantification.

Since the prostate carcinoma forms its own basement membrane when growing invasively; it can be concluded that only cells expressing Trp8 and Trp10 are involved in this phenomenon. Thus, it can be concluded that by inhibiting the expression and/or activity of these proteins an effective therapy of cancers like PCA is provided.

Thus, the present invention also relates to a pharmaceutical composition containing a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b, and a method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (uterine carcinoma) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a

therapeutically effective amount of a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b. Examples of such reagents are the above described antisense RNAs, ribozymes or inhibitors, e.g. specific antibodies. Furthermore, peptides, which inhibit or modulate the biological function of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b may be useful as therapeutical reagents. For example, these peptides can be obtained by screening combinatorial phage display libraries (Cosmix, Braunschweig, Germany) as described by Rottgen, P. and Collins, J. (Gene (1995) 164 (2): 243-250). Furthermore, antigenic epitopes of the Trp8 and Trp10 proteins can be identified by the expression of recombinant Trp8 and Trp10 epitope libraries in *E. coli* (Marquart, A. & Flockerzi, V., FEBS Lett. 407 (1997), 137-140; Trost, C., et al., FEBS Lett. 451 (1999) 257-263 and the consecutive screening of these libraries with serum of patients with cancer of the prostate or of the endometrium. Those Trp8 and Trp10 epitopes which are immunogenic and which lead to the formation of antibodies in the serum of the patients can be then be used as Trp8 or Trp10 derived peptide vaccines for immune inventions against cancer cells which express Trp8 or Trp10. Alternatively to the *E. coli* expression system, Trp8 or Trp10 or epitopes of Trp8 and Trp10 can be expressed in mammalian cell lines such as human embryonic kidney (Hek 293) cells (American Type Culture Collection, ATCC CRL 1573).

Finally, compounds useful for therapy of the above described diseases comprise compounds which act as antagonists or agonists on the ion channels Trp8, Trp9 and Trp10. It could be shown that Trp8 is a highly calcium selective ion channel which in the presence of monovalent (namely sodium) and divalent ions (namely calcium) is only permeable for calcium ions (see Example 4, below, and Figures 3A, C, E). Under physiological conditions, Trp8 is a calcium selective channel exhibiting large inward currents. This very large conductance of Trp8 channels (as well as Trp9 and Trp10a/b channels) is useful to establish systems for screening pharmacological compounds interacting with Trp-channels including high throughput screening systems. Useful high throughput screening systems are well known to the person skilled in the art and include, e.g., the use of cell lines stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and Trp10 channels in assays to detect calcium signaling in biological systems. Such systems include assays based on Ca-sensitive dyes such as aequorin, apoaequorin, Fura-2, Fluo-3 and Indo-1.

Accordingly, the present invention also relates to a method for identifying compounds which act as agonists or antagonists on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, preferably by using a system based on cells stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

For administration the above described reagents are preferably combined with suitable pharmaceutical carriers. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the tumor and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the tumor, general health and other drugs being administered concurrently.

The delivery of the antisense RNAs or ribozymes of the invention can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. By delivering these nucleic acids to the desired target, the intracellular expression of Trp8a, Trp8b, Trp10a and/or Trp10b and, thus, the level of Trp8a, Trp8b, Trp10a and/or Trp10b can be decreased resulting in the inhibition of the negative effects of Trp8a, Trp8b, Trp10a and/or Trp10b, e.g. as regards the metastasis formation of PCA.

Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic acids include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions

(mixed), micelles, liposomes and lipoplexes. The preferred colloidal system is a liposome. The composition of the liposome is usually a combination of phospholipids and steroids, especially cholesterol. The skilled person is in a position to select such liposomes which are suitable for the delivery of the desired nucleic acid molecule. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tumor. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tumors via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, i.e. tumor to be treated, the nucleic acids encoding, e.g. an antisense RNA or ribozyme can also be operably linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) *Neuron* 12, 11-24; Vidal et al.; (1990) *EMBO J.* 9, 833-840; Mayford et al., (1995), *Cell* 81, 891-904; Pinkert et al., (1987) *Genes & Dev.* 1, 268-76).

For use in the diagnostic research discussed above, kits are also provided by the present invention. Such kits are useful for the detection of a target cellular component, which is Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, wherein the presence or an increased concentration of Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts is indicative for a prostate tumor, endometrial cancer, melanoma, chorion carcinoma or cancer of the lung, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts. The probe can be detectably labeled. Such probe may be a specific antibody or specific oligonucleotide. In a preferred embodiment, said kit contains an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a-and/or anti-Trp10b-antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said kits are based on a RIA and contain said antibody marked with a radioactive isotope. In a preferred embodiment of the kit of the invention the antibody is labeled with enzymes, fluorescent compounds, luminescent compounds, ferromagnetic probes or radioactive compounds. The kit of the invention may comprise one or more containers filled with, for example, one or more probes of the invention. Associated with container(s) of the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

## EXAMPLES

The following Examples are intended to illustrate, but not to limit the invention. While such Examples are typical of those that might be used, other methods known to those skilled in the art may alternatively be utilized.

### **Example 1: Materials and Methods**

#### **(A) Isolation of cDNA clones and Northern blot analysis**

Total RNA was isolated from human placenta and prostate using standard techniques. Isolation of mRNA was performed with poly (A)<sup>+</sup>RNA - spin columns (New England Biolabs, Beverly, USA) according to the instructions of the manufacturer. Poly (a)<sup>+</sup>RNA was reverse transcribed using the cDNA choice system (Gibco-BRL, Rockville, USA) and subcloned in λ-Zap phages (Stratagene, La Jolla, USA). An human expressed sequence tag (GenBank accession number 1404042) was used to screen an oligo d(T) primed human placenta cDNA library. Several cDNA clones were identified and isolated. Additional cDNA clones were isolated from two specifically primed cDNA libraries using primers 5'-gca tag gaa ggg aca ggt gg-3' and 5'-gag agt cga ggt cag tgg tcc-3'.

cDNA clones were sequenced using a thermocycler (PE Applied Biosystems, USA) and Thermo Sequenase (Amersham Pharmacia Biotech Europe, Freiburg, Germany). DNA sequences were analyzed with an automated sequencer (Licor, Lincoln, USA).

For Northern blot analysis 5 µg human poly (A)<sup>+</sup> RNA from human placenta or prostate were separated by electrophoresis on 0.8 % agarose gels. Poly (A)<sup>+</sup> RNA was transferred to Hybond N nylon membranes (Amersham Pharmacia Biotech Europe, Freiburg, Germany). The membranes were hybridized in the presence of 50 % formamide at 42°C over night. DNA probes were labelled using [ $\alpha$ <sup>32</sup>P]dCTP and the „ready prime„ labelling kit (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Commercial Northern blots were hybridized according to the distributors instructions (Clontech, Paolo Alto, USA).

#### **(B) Construction of expression plasmids and transfection of HEK 293 cells**

Lipofections were carried out with the recombinant dicistronic eucaryotic expression plasmid pdiTRP8 containing the cDNA of Trp8b under the control of the chicken β-actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and

the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken  $\beta$ -actin promotor. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol. Cell. Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

For monitoring of the intracellular  $\text{Ca}^{2+}$  concentration human embryonic kidney (HEK 293) cells were cotransfected with the pcDNA3-TRP8b vector and the pcDNA3-GFPvector in a molar ratio of 4 : 1 in the presence of lipofectamine (Quiagen, Hilden, Germany). To obtain pcDNA3-TRP8b the entire protein coding region of TRP8b including the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research 15, 8125-8148) was subcloned into the pcDNA3 vector (Invitrogen, Groningen, Netherlands). Calcium monitoring and patch clamp experiments were carried out two days and one day after transfection, respectively.

#### **(C) Chromosomal localization of the Trp8 gene**

The chromosomal localization of the human TRP8 gene was performed using NIGMS somatic hybrid mapping panel No.2 (Coriell Institute, Camden, NJ, USA) previously described (Drwinga, H.L., Toji, L.H., Kim, C.H., Greene, A.E., Mulivor, R.A. (1993) Genomics 16, 311-314; Dubois, B.L. and Naylor, S.L. (1993) Genomics 16, 315-319).

#### **(D) In Vitro Translation, glutathione - sepharose and calmodulin agarose binding assay**

N- and C-terminal Trp8-fragments were subcloned into the pGEX-4T2 vector (Amersham Pharmacia Europe, Freiburg, Germany) resulting in glutathione-S-transferase (GST)-Trp8 fusion constructs (Fig. 4). The GST-TRP8-fusion proteins were expressed in *E. coli* BL 21 cells and purified using glutathione - sepharose beads (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

In vitro translation of human Trp8 cDNA and *Xenopus laevis* calmodulin cDNA (Davis, T.N. and Thorner, J. Proc.Natl.Acad.Sci. USA 86, 7909-7913.) was performed in the presence of  $^{35}\text{S}$ -methionine using the TNT coupled transcription/translation kit (Promega, Madison, USA). Translation products were purified by gel filtration (Sephadex G50, Amersham Pharmacia Biotech Europe, Freiburg, Germany) and equal amounts of  $^{35}\text{S}$  labeled probes were incubated for 2 h with glutathione beads bound to GST - Trp8 or calmodulin - agarose (Calbiochem) in 50 mM Tris-HCl, pH 7.4, 0.1 % Triton X-100, 150 mM NaCl in the presence of 1 mM  $\text{Ca}^{2+}$  or 2 mM EGTA. After three washes, bound proteins were eluted with SDS sample buffer, fractionated by SDS-PAGE and  $^{35}\text{S}$  labeled proteins were detected using a Phosphor Imager (Fujifilm, Tokyo, Japan).

#### **(E) Calcium measurements**

The intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) was determined by dual wavelength fura-2 fluorescence ratio measurements (Tsien, R.Y. (1988) Trends Neurosci. 11, 419-424) using a digital imaging system (T.I.L.L. Photonics, Planegg, Germany). HEK cells were grown in minimal essential medium in the presence of 10 % fetal calf serum and cotransfected with the pcDNA3-TRP8b vector and the pCDNA3-GFPvector as described above (B). Transfected cells were detected by development of green fluorescence. The cells were loaded with 4  $\mu\text{M}$  fura-2/AM (Molecular Probes, Oregon, USA) for one hour. After loading the cells were rinsed 3 times with buffer B1 (10 mM Hepes, 115 mM NaCl, 2 mM  $\text{MgCl}_2$ , 5 mM KCl, pH 7.4) and the  $[\text{Ca}^{2+}]_i$  was calculated from the fluorescence ratios obtained at 340 and 380 nm excitation wavelengths as described (Garcia, D.E., Cavalié, A. and Lux, H.D. (1994) J. Neurosci 14, 545-553).

#### **(F) Electrophysiological recordings**

HEK cells were transfected with the eucaryotic expression plasmid pdiTRP8 described in (B) and electrophysiologigal recordings were carried out one day after transfection. Single cells were voltage clamped in the whole cell mode of the patch clamp technique as described (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100; Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. 15, 6166-6171). The pipette solution contained contained (mM): 140 aspartic acid, 10 EGTA, 10 NaCl, 1  $\text{MgCl}_2$ , 10 Hepes (pH 7.2 with CsOH) or 125 CsCl, 10 EGTA, 4  $\text{CaCl}_2$  10 Hepes (pH 7.2 with CsOH). The bath solution contained (mM): 100 NaCl, 10 CsCl, 2  $\text{MgCl}_2$ , 50 mannitol, 10 glucose, 20

Hepes (pH 7.4 with CsOH) and 2 CaCl<sub>2</sub>, or no added CaCl<sub>2</sub> (-Ca<sup>2+</sup> solution). Divalent free bath solution contained (mM): 110 N-methyl-D-glucamine (NMDG). Whole cell currents were recorded during 100 msec voltage ramps from -100 to +100 mV at varying holding potentials.

#### **(G) In Situ Hybridization**

In situ hybridizations were carried out using formalin fixed tissue slices of 6 - 8 µM thickness. The slices were hydrated and incubated in the presence of PBS buffer including 10 µg / ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 0.5 h. The slices were hybridized at 37°C using biotinylated deoxy-oligonucleotides (0.5 pmol / µl) in the presence of 33 % formamide for 12 h. Furthermore the slices were several times rinsed with 2 x SSC and incubated at 25°C for 0.5 h with avidin / biotinylated horse raddish peroxidase complex (ABC, DAKO, Santa Barbara, USA). After several washes with PBS buffer the slices were incubated in the presence of biotinylated tyramid and peroxide (0.15 % w/v) for 10 min, rinsed with PBS buffer and additionally incubated with ABC complex for 0.5 h. The slices were washed with PBS buffer and incubated in the presence of DAB solution (diaminobenzidine (50µg / ml), 50 mM Tris/EDTA buffer pH 8.4, 0.15 % H<sub>2</sub>O<sub>2</sub> in N,N - dimethyl-formamide; Merck, Darmstadt, Germany). The detection was stopped after 4 minutes by incubating the slides in water. Tyramid was biotinylated by incubating NHS-LC Biotin (sulfosuccinimidyl-6-(biotinimid)-hexanoat), 2.5 mg / ml; Pierce, Rockford, USA) and tyramin-HCl (0.75 mg / ml, Sigma) in 25 mM borate buffer pH 8.5 for 12 h. The tyramid solution was diluted 1 - 5 : 1000 in PBS buffer.

#### **(H) GenBank accession numbers: TRP8a, Aj243500; TRP8b Aj243501**

#### **Example 2: Expression of TRP8 transcripts**

In search of proteins distantly related to the TRP family of ion channels, an human expressed sequence tag (EST, GenBank accession number 1404042) was identified in the GenBank database using BLAST programmes (at the National Center for Biotechnology Information (NCBI); Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J.J. (1990) Mol. Biol. 5, 403-410) being slightly homologous to the VR1 gene. Several human placenta cDNA libraries were constructed and screeened with this EST DNA as probe. Several full length

cDNA clones were identified and isolated. The full length cDNA clones encoded two putative proteins differing in three amino acids and were termed Trp8a and Trp8b (Fig. 1c, 2a, 7 and 8A). This finding was reproduced by isolating cDNA clones from two cDNA libraries constructed from two individual placentas. The derived protein sequence(s) comprises six transmembrane domains, a characteristic overall feature of trp channels and related proteins (Fig.: 1b). The sequence is closely related to the meanwhile published calcium uptake transport protein 1 (CaT1), isolated from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A. (1999) *J Biol Chem.* 6;274, 22739-22746) and to the epithelial calcium uptake channel (ECaC) isolated from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) *J Biol Chem.* 26;274, 8375-8378). Expression of Trp8a/b transcripts are detectable in human placenta, pancreas and prostate (Fig.: 5) and the size of the Northern signal (3.0 kb) corresponds with the size of the isolated full length cDNAs. In addition, a shorter transcript of 1.8 kb, probably a splice variant, is detectable in human testis. The Trp8 mRNA is not expressed in small intestine or colon (Fig.: 5) implicating that Trp8 is not the human ortholog of the rat CaT1 or rabbit ECaC proteins. To investigate whether there are other related sequences Trp8a/b derived primers (UW241, 5'-TAT GAG GGT TCA GAC TGC-3' and UW242, 5'-CAA AGT AGA TGA GGT TGC-3') were used to amplify a 105 bp fragment from human genomic DNA being 95% identical on the nucleotide level to the Trp8 sequence (data not shown). This indicates the existence of several similar sequences in humans at least at the genomic level.

**Example 3: Two variants of the Trp8 protein (Trp8a and Trp8b) arise by polymorphism**

Two variants of the Trp8 cDNA were isolated from human placenta (Fig.: 2A, 7 and 8A) which encoded two proteins which differ in three amino acids and were termed Trp8a and Trp8b. Trp8a/b specific primers were designed to amplify a DNA fragment of 458 bp of the Trp8 gene from genomic DNA isolated from human T-lymphocytes (primer pair: UW243, 5'-CAC CAT GTG CTG CAT CTA CC-3' and UW244, 5'-CAA TGA CAG TCA CCA GCT CC-3'). The amplification product contains a part of the sequence where the derived protein sequence of Trp8a comprises the amino acid valine and the Trp8b sequence methionine as well as a silent base pair exchange (g versus a) and an intron of 303bp (Fig.: 2.A, B). Both variants of the Trp8 genes (a,b) were amplified from genomic DNA in equal amounts indicating the existence of both variants in the human genome and therefore being not the

result of RNA editing (Fig.: 2B). The Trp8a gene can be distinguished from the Trp8b gene by cutting the genomic fragment of 458bp with the restriction enzyme Bsp1286I (Fig. 2B). Using human genomic DNA isolated from blood of twelve human subjects as template, the 458bp fragment was amplified and restricted with Bsp1286I. In eleven of the tested subjects only the Trp8b gene is detectable, while one subject (7) contains Trp8a and Trp8b genes (Fig.: 2D). These implicates that the two Trp8 variants arise by polymorphism and do not represent individual genes. Using Trp8 specific primers and chromosomal DNA as template, the Trp8 locus is detectable on chromosome 7 (Fig.: 2C).

#### **Example 4: Trp8b is a calcium permeable channel**

The protein coding sequence of the Trp8b cDNA was subcloned into pcDNA3 vector (Invitrogen, Groningen, Netherlands) under the control of the cytomegalovirus promotor (CMV). Human embryonic kidney (HEK 293) cells were cotransfected with the Trp8b pcDNA3 construct (pcDNA3-Trp8b vector) and the pcDNA3-GFPvector encoding the green fluorescent protein (GFP) in 4:1 ratio. The Trp8b cDNA and the cDNA of the reporter, GFP, was transiently expressed in human embryonic kidney (HEK 293) cells. The intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) and changes of  $[\text{Ca}^{2+}]_i$  were determined by dual wavelength fura-2 fluorescence ratio measurements (Fig.: 3F) in cotransfected cells which were identified by the green fluorescence of the reporter gene GFP.

Dual wavelength fura-2 fluorescence ratio measurement is a standard procedure (e.g. in: An introduction of Molecular Neurobiology (ed. Hall, Z.W.)Sinauer Associates, Sunderland, USA (1992)) using fura-2, which is a fluorescent  $\text{Ca}^{2+}$  sensitive dye and which was designed by R.Y.Tsien (e.g. Trends Neurosci. 11, 419-424 (1988) based upon the structure of EGTA. Its fluorescence emission spectrum is altered by binding to  $\text{Ca}^{2+}$  in the physiological concentration range. In the absence of  $\text{Ca}^{2+}$ , fura-2 fluoresces most strongly at an excitation wavelength of 385 nm; when it binds  $\text{Ca}^{2+}$ , the most effective excitation wavelength shifts to 345 nm. This property is used to measure local  $\text{Ca}^{2+}$  concentrations within cells. Cells can be loaded with fura-2 esters (e.g. fura-2AM) that diffuse across cell membranes and are hydrolyzed to active fura-2 by cytosolic esterases.

In the presence of 1mM  $\text{Ca}^{2+}$ , Trp8 expressing cells typically contained more than 300 nM cytosolic  $\text{Ca}^{2+}$ , while non transfected controls contained less than 100 nM  $\text{Ca}^{2+}$  ions (Fig. 3F).

When Trp8b transfected cells were incubated without extracellular  $\text{Ca}^{2+}$ , the intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) decreased to levels comparable to non transfected cells. Readdition of 1mM  $\text{Ca}^{2+}$  to the bath resulted in significant increase of the cytosolic  $[\text{Ca}^{2+}]$  in Trp8b transfected cells, but not in controls (Fig.: 3F). After readdition of  $\text{Ca}^{2+}$  ions to the bath solution, the cytosolic  $\text{Ca}^{2+}$  concentration remains on a high steady state level in the Trp8b transfected cells.

#### **Example 5: Trp8 expressing cells show calcium selective inward currents**

To characterize in detail the electrophysiological properties of TRP8, TRP8 and GFP were coexpressed in HEK293 cells using the dicistronic expression vector pdiTRP8 and measured currents using the patch clamp technique in the whole cell mode (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) *Pflugers Arch.*, 391, 85-100).

The eucaryotic expression plasmid pdiTRP8 contains the cDNA of Trp8b under the control of the chicken  $\beta$ -actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and the GFP (Prasher, D.C. et al. (1992), *Gene* 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) *Nucleic Acids Research* 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), *Gene* 8, 193-199) downstream of the chicken  $\beta$ -actin promotor. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) *Mol. Cell. Biol.* 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) *Nature* 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

In the presence of 2 mM external calcium, Trp8b transfected HEK cells show inwardly rectifying currents, the size of which depends on the level of intracellular calcium and the electrochemical driving force. The resting membrane potential was held either at -40 mV, or, to lower the driving force for calcium influx in between pulses, at + 70 mV. Current traces

were recorded in response to voltage ramps from -100 to +100 mV, that were applied every second. To monitor inward and outward currents over time, we analyzed the current size at -80 and +80 mV of the ramps. Figure 3A shows a representative trace of the current at -80 mV over time. Both at a holding potential of -40 mV or at +70 mV, the currents are significantly larger than in cells transfected with only the GFP containing vector (Fig.: 3E). Interestingly, after changing to a positive holding potential, current size in Trp8 transfected cells slowly increases and reaches steady state after approximately 70 seconds (Fig.: 3A). To determine the selectivity of the induced currents, we then perfused the cells with solutions that either contain no sodium, no added  $\text{Ca}^{2+}$  (Fig. 3A, C) or a sodium containing, but divalent ion free bath solution. To control for the effect of the solution change alone, we also perfused with normal bath (see puff in Fig. 3A). While removal of external  $\text{Ca}^{2+}$  completely abolishes the trp 8 induced currents - the remaining current being identical in size and shape to the control (Fig.: 3A, C, E), removal of external sodium has no effect (Fig.: 3E). An important hallmark of calcium selective channels (e.g. Vennekens, R., Hoenderop, G.J., Prenen, J., Stuiver, M., Willems, PHGM, Droogmans, G., Nilius, B. and Bindels, R.J.M (1999) *J. Biol. Chem.* 275, 3963-3969), is their ability to conduct sodium only if all external divalent ions, namely  $\text{Ca}^{2+}$  and magnesium are removed. To test whether the trp 8 channel conforms with this phenomenon normal bath solution was switched to a solution containing only sodium and 1 mM EGTA. As can be seen in Figure 3B and D, Trp8 transfected cells can now conduct very large sodium currents. Interestingly, immediately after the solution change, the currents first become smaller before increasing rapidly, indicating that the pore may initially still be blocked by calcium a phenomenon usually called anomalous mole fraction behaviour (Warnat, J., Philipp, S., Zimmer, S., Flockerzi, V., and Cavalié A. (1999) *J. Physiol. (Lond)* 518, 631-638). The measured outward currents of Trp8 transfected cells in normal bath solution are not significantly different from non-transfected control cells or cells which only express the reporter gene GFP. As the removal of external  $\text{Ca}^{2+}$  abolishes the Trp8 specific current, the remaining current was subtracted from the current before the solution change to obtain the uncontaminated Trp8 conductance (see inset in Fig.: 3C). As expected from the given ionic conditions (high EGTA inside, 2 mM  $\text{Ca}^{2+}$  outside), the current-voltage relationship now shows prominent inward rectification with little to no outward current.

Both the time course of the development of Trp8 currents and the size of the currents depend on the frequency of stimulation (data not shown), the internal and external  $\text{Ca}^{2+}$  concentration

and the resting membrane potential, suggesting that Trp8 calcium conductance is intricately regulated by a  $\text{Ca}^{2+}$  mediated feedback mechanisms.

#### **Example 6: $\text{Ca}^{2+}$ / calmodulin binds to the C-terminus of the Trp8 protein**

To test whether calmodulin, a prime mediator of calcium regulated feedback, is involved, first it was investigated biochemically whether Trp8 protein can bind calmodulin. Trp8 cDNA was in vitro translated in the presence of  $^{35}\text{S}$ -methionine and the product incubated with calmodulin-agarose beads. After several washes either in the presence or absence of  $\text{Ca}^{2+}$ , the beads were incubated in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis. In the presence of  $\text{Ca}^{2+}$  (1mM), but not in the absence of  $\text{Ca}^{2+}$ , Trp8 protein binds to calmodulin (Fig.: 4B).

To narrow down the binding site, two approaches were undertaken: Firstly, GST-TRP8 fusion proteins of various intracellular domains of Trp8 were constructed, expressed in *E. coli* and bound to glutathione sepharose beads. These beads were then incubated with in vitro translated  $^{35}\text{S}$ - labeled calmodulin, washed and subjected to gel electrophoresis. Secondly, truncated versions of in vitro translated Trp8 protein were used in the above described binding to calmodulin-agarose. As shown in Figure 4A, and C, fusion proteins of the N-terminal region (N1, N2) of Trp8 did not bind calmodulin, while C-terminal fragments (C1, C2, C3, C4) showed calmodulin binding in the presence of calcium (for localization of fragments within the entire Trp8 protein see Fig. 4C). Accordingly, a truncated version of in vitro translated Trp8, which lacks the C-terminal 32 amino acid residues did not bind to calmodulin-agarose (4B). We have restricted the calmodulin binding site to amino acid residues 691 to 711 of the Trp8 protein. This calmodulin binding site does not resemble the typical conserved IQ - motif of conventional myosins, but has limited sequence homology to the calcium dependent calmodulin binding site 1 of the transient receptor potential like (trpl) protein of *Drosophila melanogaster* (Warr and Kelly, 1996) with several charged amino acid residues conserved. The sequence of the calmodulin binding site of the Trp8 protein resembles a putative amphipathic  $\alpha$ -helical wheel structure with a charged and a hydrophobic site according to a model proposed by Erickson-Vitanen and De Grado (1987, Methods Enzymol. 139, 455-478.).

**Example 7: Expression of Trp8 transcripts in human placenta and pancreas**

Several slides from a human placenta of a ten week old abort were used for in situ hybridization experiments. The in situ hybridization experiments revealed expression of Trp8 transcripts in human placenta (Fig.: 5B). Expression was detectable in trophoblasts and syncytiotrophoblasts of the placenta, but not in Langhans cells.

Trp8 transcripts are detectable in human pancreas (Fig.: 5A). Therefore Trp8 probes were hybridized to tissue sections of human pancreas. The pancreatic tissues were removed from patients with pancreas cancer. Trp8 expression is detectable in pancreatic acinar cells, but not in Langerhans islets (Fig.: 5C). No Trp8 expression was found in regions of pancreatic carcinomas (data not shown).

Furthermore, the Trp8 cDNA is not detectable in human colon nor in human kidney by in situ hybridization as well as by Northern analysis (Fig.: 5A, D). The Northern results taken together with the in situ expression data indicate that the Trp8 protein is not the human ortholog of the CaT1 and ECaC channels cloned from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A. (1999) J Biol Chem. 6;274, 22739-22746) and from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378), respectively. Trp8 is unlikely to represent the human version of CaT1 as its expression is undetectable in the small intestine and colon tissues where CaT1 is abundantly expressed. If, however, Trp8 is the human version of rat CaT1, a second gene product appears to be required for  $\text{Ca}^{2+}$  uptake in human small intestine and colon attributed to CaT1 in rat small intestine and colon.

**Example 8: Differential expression of Trp8 transcripts in benign and malign tissue of the prostate**

The Trp8 transcripts are expressed in human prostate as shown by hybridization of a Trp8 probe to a commercial Northern blot (Clontech, Palo Alto, USA) (Fig.: 5A). Trp8 transcripts were not detectable by Northern blot analysis using pooled mRNA of patients with benign prostatic hyperplasia (BPH) (Fig.: 5A, prostate\*). To examine Trp8 expression on the cellular

level, sections of prostate tissues were hybridized using Trp8 specific cDNA probes (Table 3). Expression of Trp8 transcripts is not detectable in normal prostate (n = 3), benign hyperplasia (BPH, n = 15) or prostatic intraepithelial neoplasia (PIN, n = 9) (Fig.: 6A, C, E). Trp8 transcripts were only detectable in prostate carcinoma (PCA), although with different expression levels. Low expression levels were found in primary carcinomas (2 - 10 % of the carcinoma cells, n = 8) (Fig.: 7B) . Much stronger expression was detectable in rezidive carcinoma (10 - 60 %) (Fig.: 7D, n = 6) and metastases of the prostate (60 - 90 %, n = 4) (Fig.: 7F). Thus it has to be concluded that the commercial Northern blot used in Fig.: 5A contains not only normal prostate mRNA as indicated by the distributor. According to the distributors instructions the prostate mRNA used for this Northern blot was collected from 15 human subjects in the range of 14 to 60 years of age. This prostate tissue was not examined by pathologic means. Since Trp8 expression is not detectable in normal or benign prostate, this finding implicates that the mRNA used for this Northern blot was extracted in part from prostatic carcinoma tissue. To summarize, Trp8 expression is only detectable in malign prostate and, thus, the Trp8 cDNA is a marker for prostate carcinoma. The results are summarized in Table 4.

Table 3

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

- 1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Table 4

Prostate	total	negative	positive
normal	3	3	0
BPH	15	15	0
PIN	9	9	0

carcinoma

18

1

17

**(B) Differential expression of Trp8 transcripts in benign and malign tissue of the uterus**

Moreover it could be shown that Trp8 is expressed in endometrial cancer (also called cancer of the uterus, to be distinguished from uterine sarcoma or cancer of the cervix) whereas no expression was observed in normal uterus tissue. Thus, Trp8 also is a specific marker for the diagnosis of the above cancer (Fig. 12).

**Example 9: Characterization of Trp9**

The complete protein coding sequence of Trp9 was determined (Fig. 9). Trp 9 transcripts are predominantly expressed in the human prostate and in human colon. As it could be shown by Northern blot analysis, there is no difference of the expression of TRP9 in benign prostate hyperplasia (BPH, Fig. 13, upper panel left) or prostate carcinoma (Fig. 13, upper panel right). However, Trp9 is useful as a reference marker for prostate carcinoma, i.e. can be used for quantifying the expression level of Trp8. The ratio of the expression of Trp8:Trp9 in patients and healthy individuals is useful for the development of a quantitative assay.

**Example 10: Characterization of Trp10**

The complete protein coding sequence of TRP10 (a and b) was determined by biocomputing (Fig. 10 and 11). Using a 235 bp fragment of the Trp10 cDNA as probe in Northern blot analysis TRP10 transcripts could only be detected in mRNA isolated from individuals with prostate cancer (Fig. 13, bottom panel) but not in mRNA isolated from benign tissue of the prostate (prostate BPH) nor in mRNA isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 235 bp cDNA fragment of the Trp10 cDNA was amplified using the primer pair UW248 5'-ACA GCT GCT GGT CTA TTC C-3' and UW249 5'-TAT

G TG CCT TGG TTT GTA CC-3' and prostate cDNA as template. In summary, Trp10a and Trp10b, like TRP8 are also expressed in malignant prostate tissue. So far, its expression could not be observed in any other tissue examined (see above). Thus, Trp 10a and Trp10b are also useful markers which are specific for malignant prostate tissue.

Furthermore, database searches in public databases of the national center for biological information (NCBI) revealed the existence of several expressed sequence tags (EST clones) being in part identical to the Trp10 sequence. These EST clones were originally isolated from cancer tissues of lung, placenta, prostate and from melanoma. These clones include the clones with the following accession numbers: BE274448, BE408880, BE207083, BE791173, AI671853, BE390627. The results demonstrate that cancer cells of these tissues express Trp10 related transcripts whereas no expression of Trp10 transcripts in the corresponding healthy tissues are detectable (Figure 13). Furthermore, it could be shown that in cancer cells of melanoma and prostate cancer Trp10 transcripts are expressed as shown by in situ hybridizations using 4 antisense probes (Figure 14A – E and 13K-O and Table 2, above). Furthermore, it could clearly be shown that cancer cells of these tissues expressing Trp10 transcripts also express Trp10-antisense transcripts as shown in Figure 14F-J, Figure 14P-R and Figure 14T by in situ hybridizations using 4 sense probes (Table 2, above). The in situ hybridization experiments demonstrate that detection of a subset of cancer cells derived from carcinoma of lung, placenta, prostate and melanoma is feasible using antisense as well as sense probes complementary to Trp10 transcripts or complementary to Trp10-antisense transcripts, respectively.

The foregoing is meant to illustrate but not to limit the scope of the invention. The person skilled in the art can readily envision and produce further embodiment, based on the above teachings, without undue experimentation.

**What Is claimed Is:**

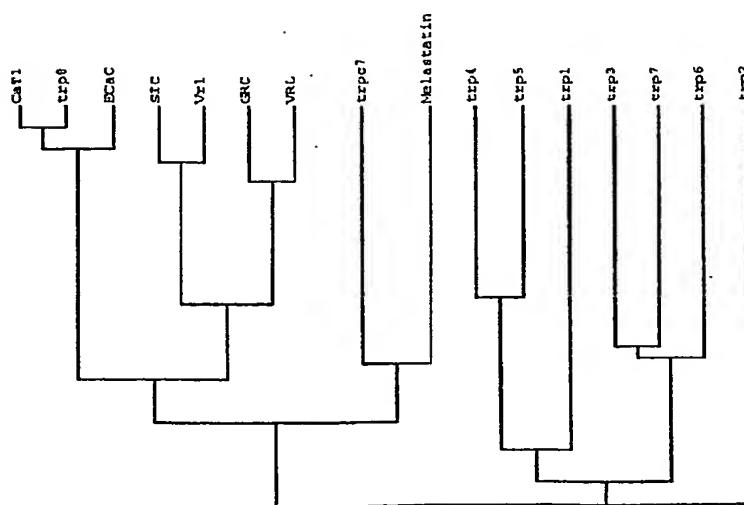
1. An isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of
  - (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11;
  - (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11;
  - (c) a nucleic acid molecule included in DSMZ Deposit No. DSM 13579, DSM 13580, DSM 13584, DSM 13581 or DSM....;
  - (d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c);
  - (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
  - (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).
2. A recombinant vector containing the nucleic acid molecule of claim 1
3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.
4. A recombinant host cell which contains the recombinant vector of claim 3.
5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
6. An isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b which is encoded by a nucleic acid molecule of claim 1.
7. A recombinant host cell that expresses the isolated protein of claim 6.

8. A method of making an isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising:
  - (a) culturing the recombinant host cell of claim 6 under conditions such that said protein is expressed; and
  - (b) recovering said protein.
9. The protein produced by the method of claim 8.
10. An antisense RNA sequence characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to said mRNA or part thereof, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
11. A ribozyme characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to and cleave said mRNA or part thereof, thus inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
12. An inhibitor characterized in that it can suppress the activity of the protein of claim 6.
13. A method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.
14. The method of claim 13, wherein the reagent is a nucleic acid.
15. The method of claim 13, wherein the reagent is an antibody.
16. The method of claim 13, wherein the reagent is detectably labeled.

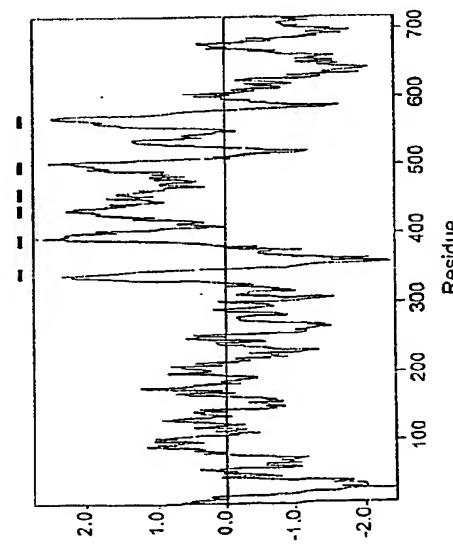
17. The method of claim 16, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
18. A method for diagnosing an endometrial cancer (carcinoma of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the Trp8a and/or Trp8a and/or trp8b encoding mRNA and detecting Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA.
19. The method of claim 18, wherein the reagent is a nucleic acid.
20. The method of claim 18, wherein the reagent is an antibody.
21. The method of claim 18, wherein the reagent is detectably labeled.
22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
23. A method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA or Trp10a and/or Trp10b related antisense RNA.
24. A method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (carcinoma of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a therapeutically effective amount of a reagent which decreases or inhibits expression of Trp8a, Trp8b, Trp10a and/or Trp10b and/or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b.
25. The method of claim 24, wherein the reagent is a nucleotide sequence comprising an antisense RNA.

26. The method of claim 24, wherein the reagent is a nucleotide sequence comprising a ribozyme.
27. The method of claim 24, wherein the reagent is an inhibitor of Trp8a, Trp8b, Trp10a and/or Trp10b.
28. The method of claim 27, wherein the reagent is an anti-Trp8a-, anti Trp8b-, anti-Trp10a-and/or anti-Trp10b antibody or a fragment thereof.
29. A diagnostic kit useful for the detection of Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts in a sample, wherein the presence of an increased concentration of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts is indicative for a prostate tumor, endometrial cancer (cancer of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts.
30. The kit of claim 29, wherein the target component to be detected is Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b and the probe is an antibody.
31. A method for identifying a compound which acts as an agonist or antagonist on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

Fig. 1 A



B



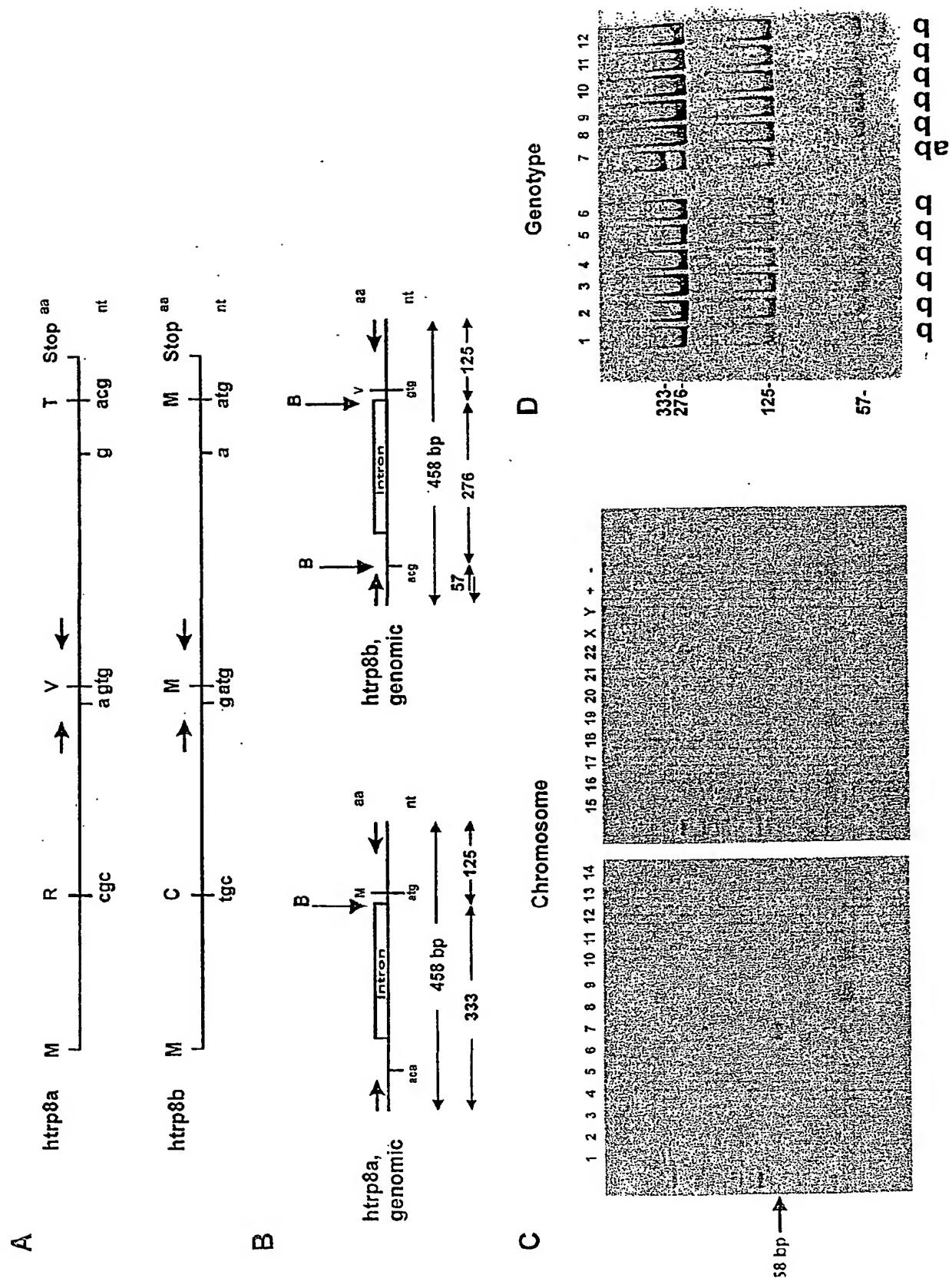


Fig. 3

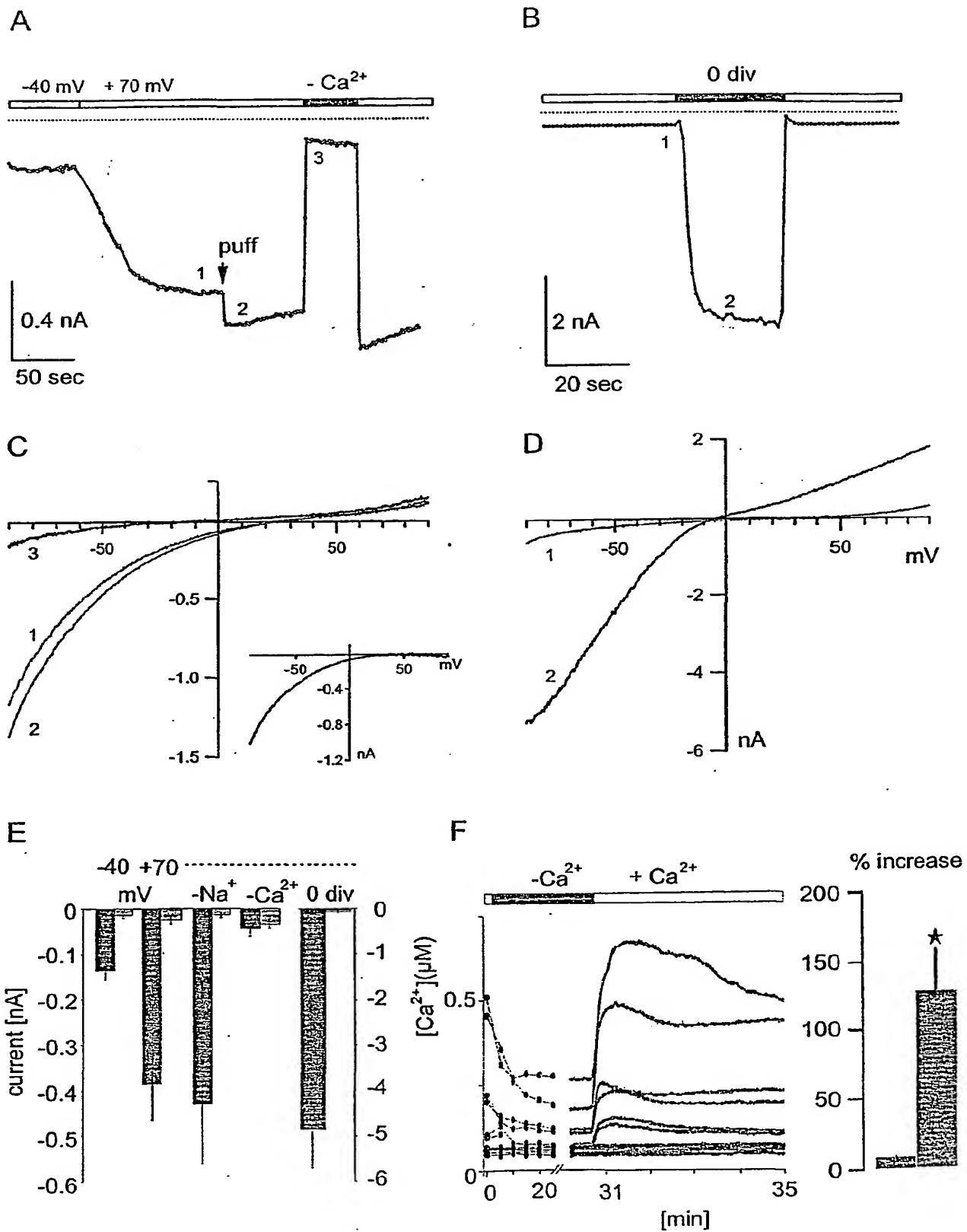


Fig. 4

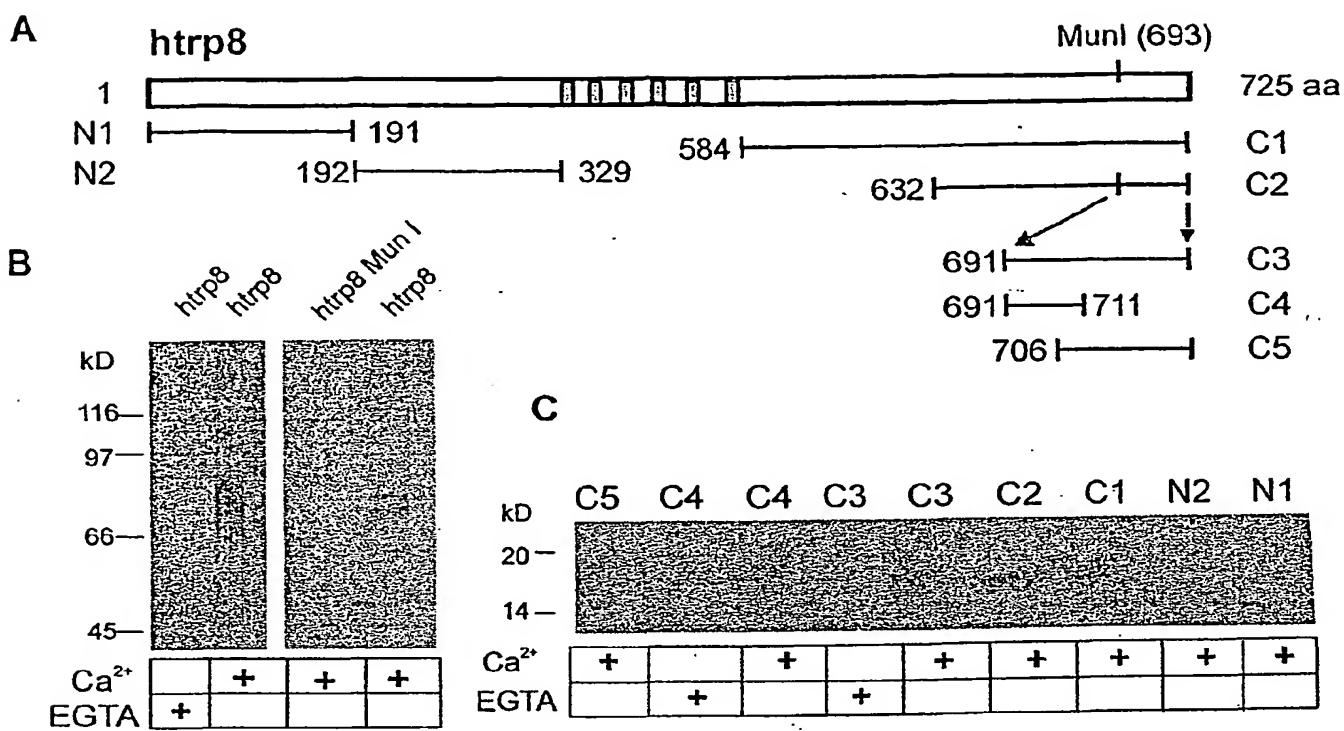
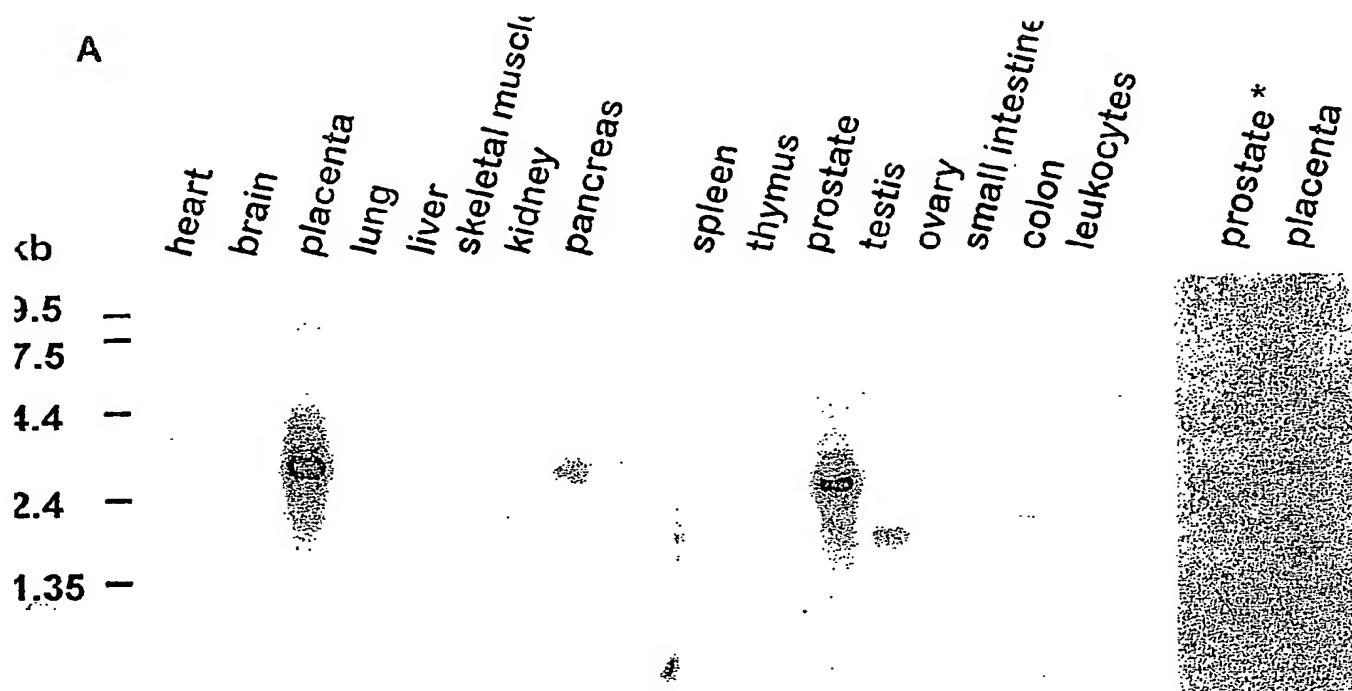
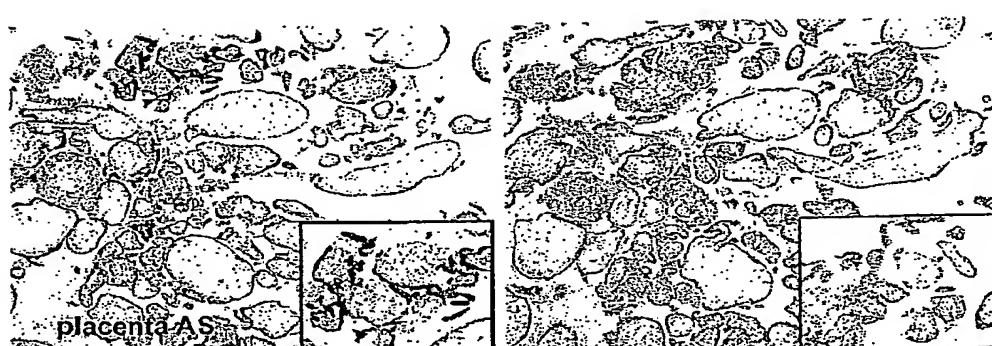


Fig. 5

A



B



C



D



Fig. 6

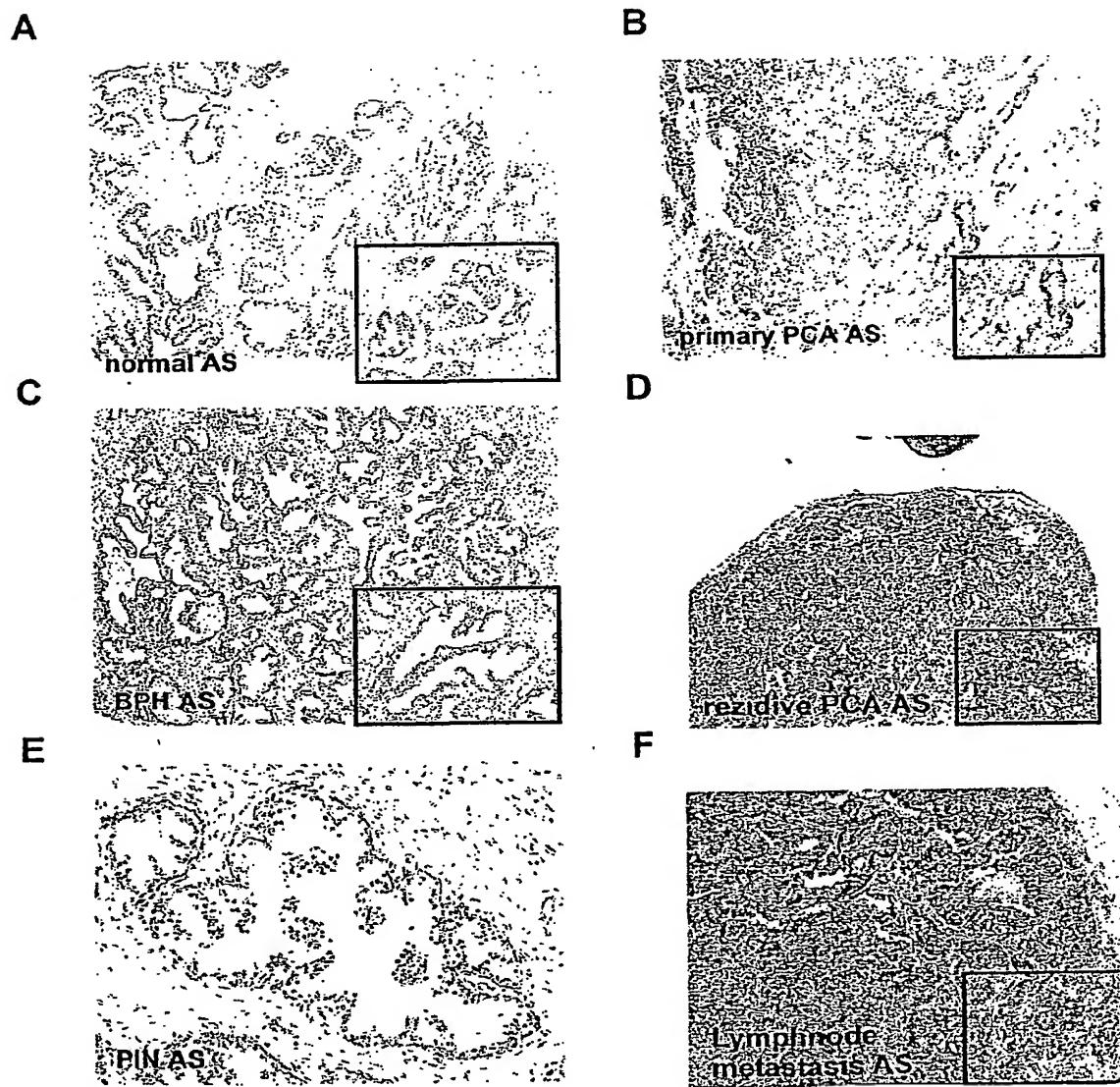


Fig. 7

10	30	50
GCCAAGTGTAAACAAACTCACAGCCCTCTCCAAACTGGCTGGGCTGCTGGAGACTCCCA		
70	90	110
AGGAACCTCGTCAGGAAGGCAGGAGACAGGAGACGGGACCTCTACAGGGAGACGGTGGGCC		
130	150	170
GGCCCTTGGGGGGCTGATGTGGCCCAAGGCTGAGTCCCGTCAGGGCTGGCCTCGGCC		
190	210	230
TCAGGGCCCCAAGGAGCCGGCCCTACACCCCATGGGTTGTCACTGCCAAGGAGAAAGG		
	M G L S L P K E K G	
250	270	290
GCTAATTCTCTGCCTATGGAGCAAGTTCTGCAGATGGTCCAGAGACGGAGTCCTGGC		
L I L C L W S K F C R W F Q R R E S W A		
310	330	350
CCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAACAGGGATCTGGAGTCTCTCTCCT		
Q S R D E Q N L L Q Q K R I W E S P L L		
370	390	410
TCTAGCTGCCAAAGATAATGATGTCAGGCCCTGAAACAAGTTGCTCAAGTATGAGGATTG		
L A A K D N D V Q A L N K L L K Y E D C		
430	450	470
CAAGGTGCACCAAGAGAGGAGCCATGGGGAAACAGCGCTACACATAGCAGCCCTCTATGA		
K V H Q R G A M G E T A L H I A A L Y D		
490	510	530
CAACCTGGAGGCCGATGGTGTGATGGAGGCTGCCCTGGTCTTTGAGCCAT		
N L E A A M V L M E A A P E L V F E P M		
550	570	590
GACATCTGAGCTCTATGAGGGTCAGACTGCACATCGCTGTTGTGAACAGAACAT		
T S E L Y E G Q T A L H I A V V N Q N M		
610	630	650
GAACCTGGTGCAGGCCCTGCTTGCCCGCAGGGCAGTGTCTTGCCAGAGCCACAGGCAC		
N L V R A L L A R R A S V S A R A T G T		
670	690	710
TGCCTTCCGCCGTAGTCCCCGCAACCTCATCTACTTGGGAGCACCTTTGTCCTTG		
A F R R S P R N L I Y F G E H P L S F A		
730	750	770
TGCCTGTCGAACAGTGAGGAGATCGTGCAGCTGCTCATGAGCATGGAGCTGACATCCG		
A C V N S E E I V R L L I E H G A D I R		
790	810	830
GGCCCAGGACTCCCTGGAAACACAGTGTACACATCCCTACCTCCAGCCCCAACAAAC		
A Q D S L G N T V L H I L I L Q P N K T		
850	870	890
CTTGCCTGCCAGATGTACAACCTGTTGCTGCTCACAGACAGACATGGGGACCACCTGCA		
F A C Q M Y N L L L S Y D R H G D R L Q		
910	930	950
GCCCCCTGGACCTCGTGCCTAACACCAGGGTCTCACCCCTTCAAGCTGGCTGGAGTGG		
P L D L V P N H Q G L T P F K L A G V E		
970	990	1010
GGGTAACACTGTGATTTCAAGCACCTGATGCAGAACGGGACACCCAGTGGACGTA		
G N T V M F Q H L M Q K R K H T Q W T Y		
1030	1050	1070
TGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCA		
G P L T S T L Y D L T E I D S S G D E Q		
1090	1110	1130
GTCCCTGCTGGAACTTATCATCACCAAGAACGGGAGGCTGCCAGATCCTGGACCA		
S L L E L I I T T K K R E A R Q I L D Q		
1150	1170	1190
GACGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAAGCGGTACGGCGCCGTACTTCTG		
T P V K E L V S L K W K R Y G R P Y F C		
1210	1230	1250
CATGCTGGGTGCCATATCTGCTGTACATCATCTGCTTCAACATGTGCTGCATCTACCG		
M L G A I Y L L Y I I C F T M C C I Y R		
1270	1290	1310

Fig. 7 / continuation 1

CCCCCCTCAAGCCCAGGACCAATAACCGACAAGCCCCGGGACAACACCCCTTTACAGCA  
 P L K P R T N N R T S P R D N T L L Q Q  
 1330 1350 1370  
 GAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGAGCT  
 K L L Q E A Y V T P K D D I R L V G E L  
 1390 1410 1430  
 GGTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAAT  
 V T V I G A I I I L L V E V P D I F R M  
 1450 1470 1490  
 GGGGGTCACTCGCTTCCTTGGACAGACCATCCTGGGGGCCATCCATGTCTCATCAT  
 G V T R F F G Q T I L G G P F H V L I I  
 1510 1530 1550  
 CACCTATGCCCTCATGGTCTGGTACCATGGTATGCCGCTCATCAGTGCCAGCGGGGA  
 T Y A F M V L V T M V N R L I S A S G E  
 1570 1590 1610  
 GGTGGTACCCATGTCCTTGCACTCGTGCTGGCTGGTCAACGTCATGTAACCGCCCG  
 V V P M S F A L V L G W C N V M Y F A R  
 1630 1650 1670  
 AGGATTCCAGATGCTAGGCCCCCTCACCATCATGATTCAAAGATGATTTGGCGACCT  
 G F Q M L G P F T I M I Q K M I F G D L  
 1690 1710 1730  
 GATGCCATTCTGCTGGCTGATGGCTGGTCATCTGGGCTTGCTTCAGCCTTCTATAT  
 M R F C W L M A V V I L G F A S A F Y I  
 1750 1770 1790  
 CATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCATGGCCCT  
 I F Q T E D P E E L G H F Y D Y P M A L  
 1810 1830 1850  
 GTTCAGCACCTTCGAGCTGTTCTTACCATCATCGATGGCCAGCCAACTAACAGTGGGA  
 F S T F E L F L T I I D G P A N Y N V D  
 1870 1890 1910  
 CCTGCCCTCATGTACAGCATCACCATGTCCTTGCACATCGCCACACTGCTCAT  
 L P F M Y S I T Y A A F A I I A T L L M  
 1930 1950 1970  
 GCTCAACCTCCTCATGCCATGATGGCGACACTCACTGGCAGGTGGCCATGAGCGGGGA  
 L N L L I A M M G D T H W R V A H E R D  
 1990 2010 2030  
 TGAGCTGTGGAGGGCCAGATTGGCCACCACGGTATGGCTGGAGCGGAAGCTGCCTCG  
 E L W R A Q I V A T T V M L E R K L P R  
 2050 2070 2090  
 CTGGCTGTGGCCTCGCTCCGGATCTGGGACGGGATATGGCTGGGGACCGCTGGTT  
 C L W P R S G I C G R E Y G L G D R W F  
 2110 2130 2150  
 CCTGGGGTGAAGACAGGAAGATCTCAACCGGCACGGATCCAACGCTACGCACAGGC  
 L R V E D R Q D L N R Q R I Q R Y A Q A  
 2170 2190 2210  
 CTTCCACACCCGGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGG  
 F H T R G S E D L D K D S V E K L E L G  
 2230 2250 2270  
 CTGTCCTTCAGCCCCCACCTGTCCTTACGCCCTCAGTGTCTCGAAGTACCTCCCG  
 C P F S P H L S L P T P S V S R S T S R  
 2290 2310 2330  
 CAGCAGTGCAATTGGGAAAGGCTCGGCAAGGGACCCCTGAGGAGAGACCTGCGTGGGAT  
 S S A N W E R L R Q G T L R R D L R G I  
 2350 2370 2390  
 AATCAACAGGGTCTGGAGGACGGGAGAGCTGGAAATATCAGATCTGACTGCGTGTCT  
 I N R G L E D G E S W E Y Q I  
 2410 2430 2450  
 CACTTCGCTCCTGGAACTTGCTCTCATTTCTGGGTGCATCAAACAAAAACAAAAACCA  
 2470 2490 2510  
 AACACCCAGAGGTCTCATCTCCAGGGCCAGGGAGAAAGAGGAGTAGCATGAACGCCAA  
 2530 2550 2570  
 GGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGCTCTGGGGCAGA

Fig. 7 / continuation 2

2590	2610	2630
GGAAGCCCAGCCAAGCACGGGGCTGGCAGGGCGTGAGGA	ACTCTCCTGTGGCCTGCTCA	
2650	2670	2690
TCACCCCTTCGACAGGAGCACTGCATGTCAGAGCACTT	AAAACAGGCCAGCCTGCTTG	
2710	2730	2750
GGCCCTCGGTCTCCACCCCAGGGTCATAAGT	GGGGAGAGAGCCCTCCCAGGGCACCCAG	
2770	2790	2810
GCAGGTGCAGGAAAGTGCAGAGCTTGTGGAAAGCGTGTGAGT	GAGGGAGACAGGAACGGC	
2830	2850	2870
TCTGGGGGTGGGAAGTGGGGCTAGGTCTTCCA	ACTCCATCTTCATAAAAGTCGTTTCG	
2890	2910	
GATCCCTAAAAAAAAAAAAAAAAAAAAAA		

MGLSLPKEKGLLILCLWSKFCRWFORRESWAQSRDEQNLQQKRIWESPILLAAKDNDVQALNKLLKYEDCKVHQRGAMGETALHIA  
 ALYDNLEAMVLMEAPELVFEPMTSELYEGQTAHLIAVVNQNMNLVRALLARRASVSARATGTAFRSPRNLIYFGEHPLSFAAC  
 VNSEEIVRLLIEHGADIRAQDLSGNNTVLHILILQPNKTFAQMNLSSYDRHGDHLQFLDLPVNHQGLTPFKLAGVEGNTVMFQH  
 LMQRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLLIELIITTKKREARQILDQTPVKEVSLKWKRYGRPYFCMLGAIYLLYIICFT  
 MCCCIYRPLKPRTRNNRTSPRDNTLLOQKLLOEAYVTPKDDIRLVGELVTIGAI I I ILLVEVPDIFRMGVTRFFGQTILGGPFHVII  
 TYAFMVLVTMVMRLISASGEVVPMMSAIVLGWCNVMFARGFQMLGPFTIMIOKMIFGDLMRFCWLMMAVVLGFASAFYIIFQTED  
 PEELGHFYDYPMALFSTFELFLTIIDGPANYNVDLPMYSITYAAFIAIATLLMLNLLIAMGDTHWRVAHERDELWRAQIVATT  
 MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRIORYAQAFHTRGSEDLKDSVEKLELGCPFSPHLSLPTPSVRST  
 SRSSANWERLRRQGTIIRRDLRGIINRGLEDGESWEYQI

Figure 8:

A)

ATGGGTTGTCACTGCCAAGGAGAAAGGGCTAATTCTCT  
 M G L S L P K E K G L I L C  
 250 270 290  
 GCCTATGGAGCAAGTTCTGCAGATGGTCCAGAGACGGGAGTCTGGGCCAGAGCCGAG  
 L W S K F C R W F Q R R E S W A Q S R D  
 310 330 350  
 ATGAGCAGAACCTGCTGCAGCAGAACAGGGATCTGGAGTCTCCTCTCCTCTAGCTGCCA  
 E Q N L L Q Q K R I W E S P L L L A A K  
 370 390 410  
 AAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACC  
 D N D V Q A L N K L L K Y E D C K V H Q  
 430 450 470  
 AGAGAGGAGCCATGGGGAAACAGCCTACACATAGCAGCCCTCTATGACAACCTGGAGG  
 R G A M G E T A L H I A A L Y D N L E A  
 490 510 530  
 CCGCCATGGTCTGATGGAGGCTGCCCGAGCTGGCTTTGAGCCATGACATCTGAGC  
 A M V L M E A A P E L V F E P M T S E L  
 550 570 590  
 TCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAAACCTGGTGC  
 Y E G Q T A L H I A V V N Q N M N L V R  
 610 630 650  
 GAGCCCTGCTTGCCTGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCAGTGCCTCCGCC  
 A L L A R R A S V S A R A T G T A F R R  
 670 690 710  
 GTAGTCCCTGCAACCTCATCTACTTGGGGAGCACCCCTTGCTCCTTGCTGCCTGTGTA  
 S P C N L I Y F G E H P L S F A A C V N

Fig. 8 / contin 1

730	750	770
ACAGTGAGGAGATCGTCGGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCAGGACT		
S E E I V R L L I E H G A D I R A Q D S		
790	810	830
CCCTGGGAAACACAGTGTACACATCCTCATCCTCCAGCCAAACAAACCTTGCTGCC		
L G N T V L H I L I L Q P N K T F A C Q		
850	870	890
AGATGTACAAACCTGTTGCTGCTCACAGACAGACATGGGACCCACCTGCAGCCCTGGACC		
M Y N L L L S Y D R H G D H L Q P L D L		
910	930	950
TCGTGCCAATCACCAAGGGTCTCACCCCTTCAAGCTGGCTGGAGTGGAGGGTAACACTG		
V P N H Q G L T P F K L A G V E G N T V		
970	990	1010
TGATGTTTCAGCACCTGATGCAGAACGGAAAGCACACCCAGTGGACGTATGGACCACTGA		
M F Q H L M Q K R K H T Q W T Y G P L T		
1030	1050	1070
CCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGATGAGCAGTCCCTGCTGG		
S T L Y D L T E I D S S G D E Q S L L E		
1090	1110	1130
AACTTATCATCACCAAGAACGGGAGGCTGCCAGATCCTGGACCAGACGCCGGTGA		
L I I T T K K R E A R Q I L D Q T P V K		
1150	1170	1190
AGGAGCTGGTGGCCCTAAGTGGAAAGCGGTACGGCGGCCGTACTCTGCTGATGCTGGGTG		
E L V S L K W K R Y G R P Y F C M L G A		
1210	1230	1250
CCATATATCTGCTGTACATCATCTGCTTCAACCATGTGCTGCATCTACCGCCCCCTCAAGC		
I Y L L Y I I C F T M C C I Y R P L K P		
1270	1290	1310
CCAGGACCAATAACCGCACGAGCCCCGGGACAACACCCCTCTTACAGCAGAAAGCTACTTC		
R T N N R T S P R D N T L L Q Q K L L Q		
1330	1350	1370
AGGAAGCCTACATGACCCCTAACGGACGATATCCGGCTGGCGGGAGCTGGTACTGTCA		
E A Y M T P K D D I R L V G E L V T V I		
1390	1410	1430
TTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAAATGGGGGTCACTC		
G A I I I L L V E V P D I F R M G V T R		
1450	1470	1490
GCTTCTTGGACAGACCATCCTGGGGCCATTCCATGTCCTCATCATCACCTATGCCT		
F F G Q T I L G G P F H V L I I T Y A F		
1510	1530	1550
TCATGGTGTGGTGACCATGGTGATGCCCTCATCAGTGCCAGCGGGAGGTGGTACCCA		
M V L V T M V M R L I S A S G E V V P M		
1570	1590	1610
TGTCCCTTGCACTCGTGCTGGCTGGTCAACGTCATGTACTTCGCCAGGGATTCCAGA		
S F A L V L G W C N V M Y F A R G F Q M		
1630	1650	1670
TGCTAGGCCCTTCACCATCATGATCAGAACATGATTTGGCGACCTGATGCGATTCT		
L G P F T I M I Q K M I F G D L M R F C		
1690	1710	1730
GCTGGCTGATGGCTGGTCATCCTGGCTTGCTCAGCCTCTATATCATCTCCAGA		
W L M A V V I L G F A S A F Y I I F Q T		
1750	1770	1790
CAGAGGACCCGAGGAGCTAGGCCACTTCTACGACTACCCATGCCCTGTTAGCACCT		
E D P E E L G H F Y D Y P M A L F S T F		
1810	1830	1850
TCGAGCTGTTCTTACCATCATGATGCCAGCCAACATCACACGTGGACCTGCCCTCA		
E L F L T I I D G P A N Y N V D L P F M		
1870	1890	1910
TGTACAGCATCACCTATGCTGCCATTGCTCATGCCACACTGCTCATGCTAACCTCC		
Y S I T Y A A F A I I A T L L M L N L L		
1930	1950	1970
TCATTGCCATGATGGCGACACTCACTGGCGAGTGGCCATGAGCGGGATGAGCTGTGGA		

Fig. 8 / conti. on 2

I A M M G D T H W R V A H E R D E L W R  
 1990 . 2010 2030  
 GGGCCCAGATTGTGGCCACCACGGTGTGCTGGAGCGGAAGCTGCCCTCGCTGCCCTGTGGC  
 A Q I V A T T V M L E R K L P R C L W P  
 2050 2070 2090  
 CTCGCTCCGGGATCTGGGACGGGAGTATGGCCTGGGAGACCGCTGGTTCTGCCGGGTTGG  
 R S G I C G R E Y G L G D R W F L R V E  
 2110 2130 2150  
 AAGACAGGGCAAGATCTAACCGGCAAGCGGATCCAACGCTACCCACAGGCCCTCCACACCC  
 D R Q D L N R Q R I Q R Y A Q A F H T R  
 2170 2190 2210  
 GGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTCCCTTC  
 G S E D L D K D S V B K L E L G C P F S  
 2230 2250 2270  
 GCCCCCCACCTGTCCCTCCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGC  
 P H L S L P M P S V S R S T S R S S A N  
 2290 2310 2330  
 ATTGGGAAAGGCTTCGGCAAGGGACCCCTGAGGGAGAGACCTGCCTGGGATAATCAACAGGG  
 W E R L R Q G T L R R D L R G I I N R G  
 2350 2370 2390  
 GTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGA  
 L E D G E S W E Y Q I \*

MGLSLPKEGLLICLWSKFCRWFQRRESWAQSRDEQNLLOOKRIWESPLLAAKDNDVQALNKLKYEDCKVHQRGAMGETALHIA  
ALYDNLEAMVLMEAAPELVFEPMTSELYEGQTALHIAVVNQNQMNLLVRALLARRASVSARATGTAERRSPCNLIYFGEHPLSFAAC  
VNSEEIVRLLIEHGADIRAQDLSGNTVLHILILQPNKTFACOMYNLLLSYDRHGDHLQPLDLVPHQGLTPFKLAGVEGNTVMFQH  
LMQKRKHTQWTYGPSTLYDLTEIDSSGDEQSLLIEITPKKREARQILDQTPVKELVSLWKRYGRPYFCMLGAIYLLYICFT  
MCCIYRPLKPRTNRTPRDNTLILQQKLLQEAQYMTPKDDIRLVGELVTVIGAIIILVEVPDIFRMRGVTRFFGQTIQILGGPFHVLI  
TYAFMVLVTVMVMLRISASGEVVPMSFALVLGWCNVMYFARGFQMLGPFTIMIQKMFQGDLMRFCWLMAVVIILGFASAFYIIFQTED  
PEELGHFYDYPMALFSTFELFLTIIDGPANYNDLPFMYSITYAAFAITIATLMLNLLIAMMGDTHWRVAHERDELWRAQIVATT  
MLERKLPLRCLWPRSGICGREYGLGDRWFLRVEDRODLNQRQIQRYAQAFHTRGSEDLDKDSVEKLELGCPSPHLSLPMPSVSRSRST  
SRSSANWERLROGTLLRDLRGIIINRGLEDGESWEYQI

B)

CAAACCTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAGGAACCTCGTCAGGAAGGCAGGAGACAGGAGACGGGAGACGGGAGACGGTGGGCCGCCCTGGGGGGCTGATGTTGGCCCCAAGGCTGAGTCCCCTGAGGGCTCTGGCCTCGGCCCTCA  
GGCCCCCAAGGAGGCCGCCCTACACCCCATGGGTTGCACTGCCCAAGGAGAAAGGCTAATTCTGCTCATGGAGCAAGTCT  
GCAGATGGTCCAGAGACGGGAGTCTGGGCCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGAGTCTCC  
CTCTCTCTAGCTGCCAAAGATAATGATGTCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTCACCCAGAGGAGC  
CATGGGGAAACAGCGCTACACATAGCAGGCCCTCTATGACAACCTGGAGGCCATGGCTGATGGAGGCTGCCCGGAGCTGG  
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCACATCGCTGTTGAAACCAGAACATGAAACCTGGTGC  
GCCCTGCTTGGCCCGAGGGCCAGTGTCTGCAAGGCCAGGGCACTGCCCTGCCGTAGTCCCCGCAACCTCATCTACTTTGG  
GGAGCACCCCTTGTCTTGTCTGCTGTCAGTGGAGATCGTGGGCTGCTATTGAGCATGGAGCTGACATCCGGGCC  
AGGACTCCCTGGCCCAACAAAACCTTGTCTGCCAGATGTCACAACCTGTCGCTTACAGACAGACATGGGACCACCTGCA  
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TGCAGAPCGGAAGCACACCCAGTGGACGTATGGACCACGTACCTCGACTCTCATGACCTCACAGAGATGACTCCT  
GAGGGAT  
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GAGCCTCAAGTGGAAAGCGGTACGGCGGCCGTACTCTGTCATGCTGGGTGCCATATATCTGTCATCATCTGCTT  
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GCTGGTAGA  
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TCATCTGGGCTTGTCTAGCCTCTATATCATCTTCCAGACAGAGGAGCCCGAGGAGCTAGGCCACTCTACGACTACCCCATG  
GCCCTGTTGACCCACCTCGAGCTGGCTTACCATCATGATGCCAGGCAACTAACACGTGGACCTGCCCTCATGTCAGCGAT  
CACCTATGTCGCTTGTGCCATCATGCCACACTGCTCATGTCACCTCCTATTGCCATGATGGGCGACACTCACTGGCGAGTGG  
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CGCTCCGGGATCTGCGGACGGGAGTATGGCTGGGGACCCCTGGTCTCTGCGGGTGGAAAGACAGGCAAGAATCT  
CAACCGGCAGCG

Fig. 8 / continuation 3

GATCCAACGCTACGCACAGGCCCTCCACACCCGGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGCTGTC  
 CCTTCAGCCCCCACCTGTCCTCAGCCCTCAGTGTCTGAGTACCTCCCGCAGCAGTGCCTGGAAAGGCTCGGCAA  
 GGGACCTGAGGAGAGACCTGCGTGGATAATCAACAGGGGCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGT  
 TCTCACTTCGCTTCGAACTTGCTCTATTTCTGGGTGCACTAAACAAACAAAACACCCAGAGGTCTCATCTCC  
 AGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAAGGAATCTACGTTGAGAATCAGTCCAGGCCGCTCATTAACCTCC  
 TCTGGGGCAGAGGAAGCCCAGGCCAAGCAGGGCTGGCAGGGCTGGAGGAACCTCTCTGTCAGCAGGCCAAG  
 GAGCACTGCATGTCAGAGCACTTTAAAACAGGCCAGGCTGTCAGGGCTGGCTCCACCCAGGGCTCATAGGGGAGAG  
 CCCTTCCAGGGCACCCAGGCAGGTGCAAGGGAGTGCAGAGCTGAGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGG  
 GTGGGAAGTGGGGCTAGGTCTTGCCAACCTCATCTCAATAAGCTGTTGGATCCCTAAAAAAAAAAAAAAAAAAAAA

c.)

CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCAAAGGAACCTCGTCAGGAAGGCAGGAGACAGGAACGGGA  
 CCTCTACAGGGAGACGGTGGGCCGCCCCCTGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGCTGGCCTCGGCCCTCA  
 GGCCCCCAAGGAGCCGCCCTACACCCCCATGGGTTGTCAGTGGCCAAGGAGAAAGGCTAATTCTCTGCCTATGGAGCAAGTCT  
 GCAGATGGTCCAGAGACGGGAGTCTGGGGCCAGAGCCGAGATGAGCAGAACCTGCTGAGCAGAAGAGGATCTGGAGTCTCC  
 CTCCCTCTAGCTGCCAAAGATAATGATGTCAGGCCCTGAACACGGTCAAGTATGAGGATTGCAAGGTGCACCCAGAGGAGC  
 CATGGGGGAAACAGCGCTACACATAGCAGGCCCTATGACAACTCTGCTCAAGTATGAGGATTGCAAGGTGCACCCAGAGGAGC  
 TCTTGAGGCCATGACATCTGAGCTCTATGAGGCTCTGACTGCCCTCATGACACCCGAGAGCTCCCTGTTCTCATCCAGGGCTAGAG  
 AAGAGGAAGAGATGGCAGCAGCTGGATCCCTGGAAATCTGAACACCCGAGAGCTCCCTGTTCTCATCCAGGGCTACCCCTGA  
 GGGAAAGAGACTGGGGTGATATGGGAGGGACCCCTGAGGATCTGGGGACAGACCCGTGACTGACAGCTGCTCTGGGGCAGG  
 TCAGACTGCACTGCACATCGCTGTTGTAACAGCAACCTGGGGCTGAGGATCTGGGGACAGACCCGTGACTGACAGCTGCTCTGCCA  
 GAGCCACAGGCACTGCTTCCGCCCTAGTCCCTGCAACCTCATCTACTTGGGGAGCACCCCTGTTGCTTGTGCTGTGAA  
 AGTGAGGAGATCGTGGGCTGCTCATGAGCATGGAGCTGACATCCGGGCCAGGACTCCCTGGCCAACAAACCTTGCCTGCC  
 AGATGTAACACCTGTTGCTGCTCATGACAGACATGGGACACCTGCAAGCCCTGGACCTCGTGCCTCATCACGGGCTCAACC  
 CCTTCAAGCTGGCTGGAGGTAACACTGATGTTGAGCAGGCTGAGAAGGGCACCTGGACCTCGTGCATGAGCTGCTATGG  
 ACCACTGACCTGACTCTATGACCTCACAGAGATGCACTCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACCA  
 AGAAGCCGGAGGCTGCCAGATCTGGGACAGACCCGGTGAAGGAGCTGGTGGCTCAAGTGGAAAGCGTACGGGCTACGGG  
 TTCTGCATGCTGGGTGCCATATCTGCTGTACATCATCTGCTTCAACATGTGCTGCACTACCGCCCCCTCAAGCCAGGACAA  
 TAACCGCACGAGCCCCGGGACAACACCCCTTACAGCAGAAGCTACTTCAGGAAGGCTACATGACCCCTAAGGACGATATCCGG  
 TGGTGGGGAGCTGGTACTGTCATTGGGCTATCATCATCTGCTGGTAGAGGTTCCAGACATCTCAGAATGGGGTCACTCG  
 TTCTTGAGACACCATTGCTGGGGCCATTCCATGTCCTCATCATCACCTATGCTTCACTGGTGTGGTGGCTGACCTATG  
 GCTCATCAGTGCAGCGGGGAGGTGGTACCCATGTCCTTGCACTGTCGTGGCTGGTGGCAACGTCATGTAACCTCGGCC  
 TCCAGATGCTAGGCCCTTCACCATCATGATTGAGAAGATGATTTTGGGACCTGATGCGATTGCTGCTGATGGCTG  
 ATCTGGGCTTTGCTTAGACAGAGGACCCCGAGGGCTAGGGCACTTCTACGACTACCCCATGGGCCCTGTTGAGCACCT  
 GGCTTACCATCATGATGGGCCAGCCAACACTAACGTTGACCTGCTGGCTGACCTGAGGACATGGTGTGGGATG  
 TCGCCACACTGCTCATGCTCAACCTCTCATGGCATGATGGCGACACTCACTGGCGAGTGGGCCATGAGGGGATG  
 AGGGCCAGATTGTTGGGACCGGTGATGTCGGAGCGGAAGCTGCCCTGGCTGGCTGCTGGCTCGCTCCGGGATCTGG  
 GTATGCCCTGGGAGACCGCTGGTCTGCGGCTGCAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCT  
 ACCGACAGGCTCCACCCGGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTC  
 CCTCATGCCCTCAGTGTCTGAGTACCTCCCGCAGCAGTGCCTGGGAAAGGCTTGGCAAGGGACCCCTGAGGAGACCT  
 TGGGATAATCAACAGGGGCTGGCAGGGCGTGGAGGAACACTCTCTGCTCATCACCCCTCCAGGGCTAAG  
 GCTCTCATTTCTGGGTGCACTAAACAAACAAAACACCCAGAGGTCTCATCTCCAGGGGGCCAGGGAGAAAGAGGAGT  
 AGCATGAGGCCAAGGAATGTACGTTGAGAATCACTGTCCTCCAGGGCTCATGAGGAGGACTGCACT  
 CAAGCACGGGGCTGGCAGGGCGTGGAGGAACACTCTCTGCTCATCACCCCTCCAGGGGACTGCACT  
 TAAAAACAGGGCAGGCTGCTGGGGCCATTGGGTCTCCACCCCAAGGGTCAATAAGTGGGGAGAGGAGG  
 GTGCAGGGAAAGTGCAGAGCTGTGAGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTGAGGGGCTG  
 GTGGGAAGTGGCTAGGTCTTGCAACTAAAGCTGTTGGATCCCTAAAAAAAAAAAAA

d.)

CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCAAAGGAACCTCGTCAGGAAGGCAGGAGACAGGAACGGGA  
 CCTCTACAGGGAGACGGTGGGCCCTGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGCTGGCCTCGGCCCTCA  
 GGCCCCCAAGGAGCCGCCCTACACCCCCATGGGTTGTCAGTGGCCAAGGAGAAAGGCTAATTCTCTGCCTATGGAGCAAGTCT  
 GCAGATGGTCCAGAGACGGGAGTCTGGGGCCAGAGCCGAGATGAGCAGAACCTGCTGAGCAGAAGAGGATCTGGAGTCTCC  
 CTCCCTCTAGCTGCCAAAGATAATGATGTCAGGCCCTGAAACAGGTTGCTCAAGTATGAGGATTGCAAGGTGCACCCAGAGGAGC  
 CATGGGGAAACAGCGCTACACATAGCAGGCCCTATGACAACACTGGAGGCCCATGGTGTGCTGAGGGCTGCCCCGGAGCTGG  
 TCTTGAGGCCATGACATCTGAGCTCTATGAGGGTCAAGTGCAGACTGCACATGCTGTTGAGGAAACATGAACCTGGTGC  
 GCCCTGCTGCCGCCAGGGCCAGTGTCTGCCCCAGGCCACAGGACTGCCCTGGCCGCTAGTCCCGCAACCTCATCTACTTGG

Fig. 8 / continuation

AAACACAGTGTACACATCCTCATCCTCCAGCCCCAACAAAACCTTGCCTGCCAGATGTACAACCTGTTGCTGTCCCTACGACAGAC  
 ATGGGGACCACCTGCAGCCCCCTGGACCTCGTGCCTAACACCCAGGGTCTCACCCCTTCAAGCTGGCTGGAGTGGAGGGTAACACT  
 GTGATGTTTACGACACCTGATGCAGAACGGAAAGCACACCCAGTGGACGTATGGACACTGACCTCGACTCTATGACCTCACAGA  
 GATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAACCTTATCATCACCAAGAACGGGAGGCTGCCAGATCCTGGACCAGA  
 CGCCGGTGAAGGAGCTGGTGAGGCTCAAGTGGAAAGCGGTACGGGGGGCGTACTCTGCTGCTGGGATATATCTGCTGTAC  
 ATCATCTGCTTACCATGTGCTGCATCTACCGCCCCCTCAAGCCAGGACCAATAACCGACAAGCCCCCGGACACACACCCTCTT  
 ACAGCAGAACGCTACTTCAGGAAGGCTACGTGACCCCTAACGGACGATATCCGGCTGGCTGGGGAGCTGGTACTGTCATTGGGCTA  
 TCATCATCTGCTGGTAGAGGTTCCAGACATCTCAGAATGGGGTCACTCGCTTCTTGGACAGACCATCTTGCCCCCATTC  
 CATGCTCTCATCATCACCTATGCCCTCATGGTCTGGTACCATGGTATGCCGCTCATCAGTGCAGGGGAGGAGGAGGTTACCCAT  
 GTCTTTCGACTCGTGGGCTGGTGAAACGTCATGTCATCTGGGCTGGGATTCCAGATGCTAGGCCCCCTCACCATCATGATTC  
 AGAAGATGATTTTGGGACCTGATGCCGATTCTGCTGGCTATGGCTGTTGCTCATCTGGGCTTCTGCTCAGCCTCTGAGCTGGCTTACCAT  
 TTCCAGACAGAGGACCCCAGGGAGCTAGGCCACTTCTACGACTACCCATGCCCTGTTGCTCAGCACCTCGAGCTGGCTTACCAT  
 CATCGATGGCCCAGCCAACATACAACGTTGACCTGCCCTCATGTCAGCATCACCTATGTCGCCCTGCCATCATGCCACACTGC  
 TCATGCTCAACCTCTCATGCCATGATGGCGACACTCACTGGCAGTGGCCATGAGCGGGATGAGCTGTGGAGGGCCAGATT  
 GTGGCCACACGGTATGCTGGAGCGGAAGCTGCTCGCTCGCTGTGGCCTCGCTCCGGATCTGCCGACGGGAGTATGGCCTGG  
 GGACCGCTGGTCTCTGCCGGTGGAAAGACAGGCAAGATCTCAACCCAGCGGATCCAACGCTACGACAGGCCCTCCACACCCGG  
 GCTCTGAGGATTGGACAAAGACTCAGTGGAAAAGACTAGAGCTGGGCTGTCCTCAGCCCCACCTGTCCTTCCACGCCCTCA  
 GTGCTCGAAGTACCTCCCGAGCAGTGCAATTGGAAAGGCTCGCAAGGGACCTGAGGAGAGACCTGCGTGGATAATCAA  
 CAGGGTCTGGAGGACGGGAGAGCTGGAAATATCAGATCTGACTGCGTGTCTCACTTCGCTCTGGAACCTGCTCTCATTTTC  
 CTGGGTGATCAAACAAAACAAAACACCAGGGCTCATCTCCAGGCCCCAGGGAGAAAGAGGAGTACGATGAAACGCC  
 AAGGAATGTAAGTGGAAATCATGCTCCAGGCTGCTTACTCTCAGCTCTGGGAGAGGAGGAGGAGGAGGAGGAGGAGG  
 TGGCAGGGCGTGGAGGAACTCTCTGTCATCACCCCTCCAGGGACTGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG  
 AGCCTGCTTGGGCCCCCTGGCTCTCCACCCAGGGCTATAAGTGGGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG  
 GCAGAGCTTGTGGAAAGGGTGTAGTGAGGGAGACAGGAACGCCCTGTCGGGGTGGGAAGTGGGCTAGGTCTTGCAACTCCATCT  
 TCAATAAGTGTGTTTGGATCCCTAAAAAAAAAAAAAAAAAAAAA

e.)

CACACATGGGGCTCCCAGGAGTCCCCAGGACCTCGTGTGGCTCTGAAATCTATGCTCTCAATCCGTGTCCCACAGAAC  
 CATATAACCCACCTCTGTAAATGCCAGGAGCATGGGGAAACAGCGCTACACATAGCAGCCCTATGACAACCTGGAGGCCG  
 CCATGGTGTGATGGAGGCTGCCCGGAGCTGGCTTTGAGCCATGACATCTGAGCTATGGAGGGTGGAGGCCACGGCTCG  
 GGGTGAAGAGCAGGAGTGAAGTGGTTGGTATTCAAGTCAGTCTGTCAGTCCCTGACTGCCATCACTTGAAACGCCGCCCCCTGAAATGCCAGGG  
 CCTACTCTTTCTCTCTGCTCTCCCTCCGTGTCAGTCCCTGACTGCCATCACTTGAAACGCCGCCCCCTGAAATGCCAGGG  
 GCCTAGAGAAGAGGAAAGAGATGGCAGCAGCTGGATCCCTGGAACTCTGAAACACCCAGAGGCTCCCTGTTCTCCATCCCAGGCT  
 ACCCCCTGAGGGAAAGAGACTAGGGGTGCAATATGGGAGGGACCCCTGCAAGGATCTTAGGGGACAGACCGTGACTGACAGCTGCT  
 CTGGGCCAGGGTCAAGTGCAGTCACATCGCTGTGTAACCAAGAACATGAAACCTGGTGGAGGCCCTGCTTGCCCCCAGGG  
 GTCTCTGCCAGGCCACAGGCACTGCCCTCCGGCGTAGTCCCTGCAACCTCATCTACTTGGGAGGACCCCTTGTCTTGTG  
 CTGTTGAAACAGTGGAGGAGATCGTGGGCTGTCATTGAGCATGGAGCTGACATCCGGGCCCAGGACTCCCTGGATGTACAACCTG  
 TTGCTGCTTACGACAGACATGGGACCCCTGCAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG  
 TGGAGTGGAGGGTAACACTGTGATGTTGAGCAGCTGATGCAAGAGGAGACACCCAGTGGACGTATGGACCAACTGACCTCGA  
 CTCTCTATGACCTCACAGAGATGACTCCCTAGGGGAGTGGCAAGTCCCTGTCAGAATGGGGTCACTCGTTCTTGGACAGAC  
 CGCCAGATCTGGACCAGACGCCGTGAAGGAGCTGGTGGAGCTCAAGTGGAAAGCGGTACGGGCGCGTACTCTGCTGATGCTGG  
 TGCCATATATCTGCTGTCATCATCTGCTTACCATGTCATCTGCTGCACTTACGGGCCCCCTCAAGGCCAGGACCAATAACCGCAGGCC  
 CCCGGACAACACCCCTCTACAGCAGAACGCTACTCAGGAAGGCTACATGACCCCTAACGGACGATATCCGCTGGTGGAGGAGC  
 GTGACTGTCATTGGGCTATCATCATCTGCTGGTAGAGGTTCCAGACATCTCAGAATGGGGTCACTCGTTCTTGGACAGAC  
 CATCTTGGGGCCCATTCCATGTCCTCATCATCACCTATGCCCTCATGGTGTGGTGGAGCTGAGCTGGCTCATCTGCTGG  
 GCGGGAGGGGGTACCCATGTCCTTGCACCTGCTGGTGGAGCTGGTGGAGCTGAGCTGGCTCATCTGCTGGTGGAGCTGG  
 CCCTTACCATCATGATTGAGAACGATGATTTTGGCAGGACTGTCATGGCTGAGCTGGCTGTCATCTGCTGGTGGTGG  
 TTCAGCCTCTATATCATCTTCCAGACAGAGGACCCAGGGAGCTAGGCCACTCTACGACTACCCCATGCCCTGTCAGCACCT  
 TCGAGCTGGCTTACCATCATGATGCCAGCCAACATACAACGTTGACCTGCCCTCATGTCAGCATCACCTATGCTGCCCTT  
 GCCATCATGCCACACTGCTCATGCTAACCTCTCATTGCCATGATGGCGACACTCACTGGCGAGTGGCCATGAGCGGGATG  
 GCTGTTGGAGGGCCCAGATTGTCCTGGCACCACGGTGTAGCTGGAGCGGAAGCTGCTCGCTGCTGGCTCGCTCCGGGATCTGCG  
 GACGGGAGTATGGCTGGAGACCGCTGGTCTGCGGGTGGAGACAGGCAAGATCTCACCGGCAGCGGATCCAACGCTACCG  
 CAGGCCCTCACACCCGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAGACTAGAGCTGGGCTGTCCTTCACTGCC  
 GTCCCTTCCATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGGCAATTGGAAAGGCTTGGCAAGGGACCCCTGAGGAGAG  
 ACCTGCGTGGATAATCAACAGGGGCTGGAGGACGGGGAGAGGCTGGGAATATCAGATCTGACTGCGTGTCTCATCTGCTTCT  
 GGAACCTGCTCTCATTTCTGGGTGCAATCAACAAAACAAAACACCCAGAGGCTCATCTCCCAAGGCCAGGGAGGAGA  
 GAGGAGTAGCATGAAACGCCAAGGAATGTACGTTGAGAATCACTGCTCCAGGCCGTCATTACTCTTCACTGCTCTGGGGAGAG  
 CCCAGGCCAAGCACGGGCTGGCAGGGCTGGAGGAACCTCTCTGTCATCACCCCTCCGACAGGAGCAGTGCATGTCAG  
 AGCACTTTAAAACAGGCCAGGCCGTTGGGCCCCCTGGTCTCACCCAGGGTCAATAGTGGGAGAGGAGGCCCTTCCAGGGCACC

Fig. 8 / continuation 5

CAGGCAGGTGCAGGGAAAGTGCAGAGCTTGTGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGCTA  
GGTCTTGCCAACTCCATCTTCATAAAAGTCGTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAA

Figure 9:

A.

10	30	50
CGGGGCCCTGGGCTGCAGGAGGTTGCGGCCGCCGGCAGCATGGTGGTGCCGGAGAAGG		
	M V V P E K E	
70	90	110
AGCAGAGCTGGATCCCCAAGATCTTCAAGAAGAAGACCTGCACGACGTTCATAGTTGACT		
Q S W I P K I F K K K T C T T F I V D S		
130	150	170
CCACAGATCCGGGAGGGACCTTGTGCCAGTGTGGGCGCCCCGGACCGCCACCCCGCAG		
T D P G G T L C Q C G R P R T A H P A V		
190	210	230
TGGCCATGGAGGATGCCCTCGGGCAGCCGTGGTACCGTGTGGGACAGCGATGCACACA		
A M E D A F G A A V V T V W D S D A H T		
250	270	290
CCACGGAGAACCCACCGATGCCAACGGATCCAGCTGGACTTCACGGGGCCGGCGCAAGC		
T E K P T D A Y G E L D F T G A G R K H		
310	330	350
ACAGCAATTCTCCGGCTCTGACCGAACGGATCCAGCTGCAGTTATAGTCTGGTCA		
S N F L R L S D R T D P A A V Y S L V T		
370	390	410
CACGCACATGGGCTTCCGTGCCCGAACCTGGTGTGCAGTGCCTGGGGATCGGGG		
R T W G F R A P N L V V S V L G G S G G		
430	450	470
GCCCCGTCCTCCAGACCTGGCTGCAGGACCTGCTGCCTGGCTGGTGCCTGGCTGCC		
P V L Q T W L Q D L L R R G L V R A A Q		
490	510	530
AGAGCACAGGAGCTGGATTGTCACTGGGGCTGCAACACGGGATCGGCCGATGTTG		
S T G A W I V T G G L H T G I G R H V G		
550	570	590
GTGTGGCTGTACGGGACCATCAGATGGCCAGCACTGGGGCACCAAGGTGGTGGCCATGG		
V A V R D H Q M A S T G G T K V V A M G		
610	630	650
GTGTGGCCCCCTGGGGTGTGGTCCGGATAGAGAACCCCTCATCAACCCAAAGGGCTCGT		
V A P W G V V R N R D T L I N P K G S F		
670	690	710
TCCCTGCGAGGTACCGGTCGGCGCGGTGACCCGGAGGACGGGGTCCAGTTCCCTGGACT		
P A R Y R W R G D P E D G V Q F P L D Y		
730	750	770
ACAACTACTCGGCCCTTCTCCTGGTGGACGACGGCACACACGGCTGCCTGGGGCGAGA		
N Y S A F F L V D D G T H G C L G G E N		
790	810	830
ACCGCTCCGCTTGCCTGGAGTCCTACATCTCACAGCAGAACACGGGCTGGAGGGGA		
R F R L R L E S Y I S Q Q K T G V G G T		
850	870	890
CTGGAAATTGACATCCCTGCTCTGCTCCCTGATTGATGGTGTGAGAAGATGTTGACGC		
G I D I P V L L L I D G D E K M L T R		
910	930	950
GAATAGAGAACGCCACCCAGGCTCAGCTCCATGTCCTCGTGGCTGGCTCAGGGGAG		
I E N A T Q A Q L P C L L V A G S G G A		
970	990	1010
CTGCGGACTGCCCTGGCGAGACCCCTGGAAGACACTCTGGCCCCAGGGAGTGGGGAGCCA		
A D C L A E T L E D T L A P G S G G A R		
1030	1050	1070
GGCAAGGCCAGGCCAGATCGAATCAGGCCTTCTTCCAAAGGGACCTTGAGGTCC		

Fig. 9 / continuing 1

Q G E A R D R I R R F F P K G D L E V L  
 1090 1110 1130  
 TGCAGGGCCCAGGTGGAGAGGATTATGACCCGGAAAGGAGCTCCTGACAGTCTATTCTTCTG  
 Q A Q V E R I M T R K E L L T V Y S S E  
 1150 1170 1190  
 AGGATGGGTCTGAGGAATTGAGACCATAGTTTGAAGGCCCTGTGAAGGCCTGTGGGA  
 D G S E E F E T I V L K A L V K A C G S  
 1210 1230 1250  
 GCTCGGAGGCCTCAGCTTACCTGGATGAGCTGCGTTGGCTGTGGCTTGGAAACCGCGTGG  
 S E A S A Y L D E L R L A V A W N R V D  
 1270 1290 1310  
 ACATTGCCAGAGTGAACTCTTCGGGGGACATCCAATGGCGTCCCTCATCTCGAAG  
 I A Q S E L F R G D I Q W R S F H L E A  
 1330 1350 1370  
 CTTCCCTCATGGACGCCCTGCTGAATGACCGCCCTGAGTTCGTGCCTGCTCATTTCCC  
 S L M D A L L N D R P E F V R L L I S H  
 1390 1410 1430  
 ACGGCCTCAGCCTGGGCCACTTCTGACCCGATGCGCTGGCCAACCTCTACAGCGCGG  
 G L S L G H F L T P M R L A Q L Y S A A  
 1450 1470 1490  
 CGCCCTCCAACTCGCTCATCCGCAACCTTTGGACCGCGTCCCAAGCGCAGGCACCA  
 P S N S L I R N L L D Q A S H S A G T K  
 1510 1530 1550  
 AAGCCCCAGCCCTAAPAGGGGGAGCTCGGGAGCTCCGGCCCCCTGACGTGGGCATGTGC  
 A P A L K G G A A E L R P P D V G H V L  
 1570 1590 1610  
 TGAGGATGCTGGGAAGATGTGCGCGCCGAGGTACCCCTCCGGGGGGCCCTGGGACCC  
 R M L L G K M C A P R Y P S G G A W D P  
 1630 1650 1670  
 CTCACCCAGGCCAGGGCTTCGGGGAGAGCATGTATCTGCTCTCGGACAAGGCCACCTCGC  
 H P G Q G F G E S M Y L L S D K A T S P  
 1690 1710 1730  
 CGCTCTCGCTGGATGCTGGCCTCGGGCAGGCCCTGGAGCGACCTGCTTCTGGCAC  
 L S L D A G L G Q A P W S D L L L W A L  
 1750 1770 1790  
 TGTTGCTGAACAGGGCACAGATGCCATGTACTTCTGGAGATGGGTTCCAATGCAGTT  
 L L N R A Q M A M Y F W E M G S N A V S  
 1810 1830 1850  
 CCTCAGCTCTGGGGCCTTGGCTCGTCCGGGTATGCCACGCCCTGGAGGCTGACGCTG  
 S A L G A C L L L R V M A R L E P D A E  
 1870 1890 1910  
 AGGAGGCAGCACGGAGGAAAGACCTGGCCTCAAGTTGAGGGATGGCGTTGACCTCT  
 E A A R R K D L A F K F E G M G V D L F  
 1930 1950 1970  
 TTGGCGAGTGCTATCGCAGCAGTGAGGTGAGGGCTGCCGCCCTCCTCCGCTGCTGCC  
 G E C Y R S S E V R A A R L L L R R C P  
 1990 2010 2030  
 CGCTCTGGGGGATGCCACTTGCTCCAGCTGCCATGCAAGCTGACGCCGTGCCCTCT  
 L W G D A T C L Q L A M Q A D A R A F F  
 2050 2070 2090  
 TTGCCAGGATGGGGTACAGTCTGCTGACACAGAAGTGGTGGGGAGATATGCCAGCA  
 A Q D G V Q S L L T Q K W W G D M A S T  
 2110 2130 2150  
 CTACACCCATCTGGGCCCTGGTCTCGCCTCTGGCCCTCCACTCATCTACACCCGCC  
 T P I W A L V L A F F C P P L I Y T R L  
 2170 2190 2210  
 TCATCACCTTCAGGAAATCAGAAAGAGGAGCCCACACGGGAGGAGCTAGAGTTGACATGG  
 I T F R K S E E E P T R E E L E F D M D  
 2230 2250 2270  
 ATAGTGTCAATTAGGGGAAGGGCCTGTCGGGACGGCGGACCCAGCCGAGAAGACGCC  
 S V I N G E G P V G T A D P A E K T P L  
 2290 2310 2330

Fig. 9 / continuation 2

TGGGGGTCCC CGCC CAGTCGGGCCGTCCGGTTGCTGCGGGGGCCGCTGCGGGGGCGCC  
 G V P R Q S G R P G C C C G G R C G G R R  
 2350 2370 2390  
 GGTGCCTACGCCGCTGGTCCACTCTGGGGCGTGCGGGTGACCATCTCATGGCAACG  
 C L R R W F H F W G V P V T I F M G N V  
 2410 2430 2450  
 TGGTCAGCTACCTGCTGTTCTGCTGCTTTCTCGCGGGTGCTGCTCGTGGATTCCAGC  
 V S Y L L F L L F S R V L L V D F Q P  
 2470 2490 2510  
 CGCGCCGCCGGCTCCCTGGAGCTGCTGCTCTATTCCTGGCTTCACGCTGCTGTGCG  
 A P P G S L E L L Y F W A F T L L C E  
 2530 2550 2570  
 AGGAACCTGCCAGGGCCTGAGCGGAGGCCGGCAGCTCGCCAGCGGGGCCGGCG  
 E L R Q G L S G G G G S L A S G G P G P  
 2590 2610 2630  
 CTGGCCATGCCACTGAGCCAGGCCCTGCGCCTCTACCTCGCCAGCTGGAAACAGT  
 G H A S L S Q R L R L Y L A D S W N Q C  
 2650 2670 2690  
 GCGACCTAGTGGCTCTACCTGCTTCTGGCGTGGCTGCCGGCTGACCCGGTT  
 D L V A L T C F L L G V G C R L T P G L  
 2710 2730 2750  
 TGTACCACTGGCCGACTGCTCTGCACTGACTTCATGGTTTCACGGTGGCTGC  
 Y H L G R T V L C I D F M V F T V R L L  
 2770 2790 2810  
 TTCACATCTCACGGTCAACAAACAGCTGGGCCAAGATCGTCATCGTGAGCAAGATGA  
 H I F T V N K Q L G P K I V I V S K M M  
 2830 2850 2870  
 TGAAGGACGTGTTCTTCTCCCTCTCCCTCGGGCTGTGGCTGGTAGCCTATGGCGTGG  
 K D F F F F L F L G V W L V A Y G V A  
 2890 2910 2930  
 CCACGGAGGGCTCCTGAGGCCACGGGACAGTGAATCCCAAGTATCCCTGCCCGCT  
 T E G L L R P R D S D F P S I L R R V F  
 2950 2970 2990  
 TCTACCGTCCCTACCTGCAGATCTCGGGCAGATCCCCAGGAGGACATGGACGTGGCCC  
 Y R P Y L Q I F G Q I P Q E D M D V A L  
 3010 3030 3050  
 TCATGGAGCACAGCAACTGCTCGGAGCCGGCTCTGGGACACCCCTCTGGGCC  
 M E H S N C S S E P G F W A H P P G A Q  
 3070 3090 3110  
 AGCCGGCACCTGCCCTCCCACTGCAACTGGCTGGTGGCTGCTCCCTCGTCATCT  
 A G T C V S Q Y A N N L V V L L L V I F  
 3130 3150 3170  
 TCCTGCTCGTGGCAAACATCCTGCTGGTCAACTTGCTCATTGCATGTTAGTACACAT  
 L L V A N I L L V N L L I A M F S Y T F  
 3190 3210 3230  
 TCGGCAAAGTACAGGGCAACAGCGATCTACTGGAAGGCGAGCGTTACCGCCTCATCC  
 G K V Q G N S D L Y W K A Q R Y R L I R  
 3250 3270 3290  
 GGGAAATTCCACTCTGGCCCGCGCTGGCCCGCCCTTATCGTCATCTCCCACTTGC  
 E F H S R P A L A P P F I V I S H L R L  
 3310 3330 3350  
 TCCTGCTCAGGCAATTGTCAGGCAGCCCGAGCCCCCAGCCGCTCTCCCGGCCCTCG  
 L L R Q L C R R P R S P Q P S S P A L E  
 3370 3390 3410  
 AGCATTTCGGGTTTACCTTCTAAGGAAGGCCAGCGGAAGCTGCTAACGTGGGAATCGG  
 H F R V Y L S K E A E R K L L T W E S V  
 3430 3450 3470  
 TGCATAAGGAGAACTTCTGCTGGCACGCCAGGGACAAGCGGGAGAGCGACTCCGAGC  
 H K E N F L L A R A R D K R E S D S E R  
 3490 3510 3530  
 GTCTGAAGCGCACGTCCAGAAGGTGGACTTGGCACTGAAACAGCTGGACACATCCGCG  
 L K R T S Q K V D L A L K Q L G H I R E

Fig. 9 / continu<sup>r</sup> 7 3

3550	3570	3590
AGTACGAACAGCGCTGAAAGTGTGGAGCGGGAGGTCCAGCAGTGTAGCCCGTCTGG		
Y E Q R L K V L E R E V Q Q C S R V L G		
3610	3630	3650
GGTGGGGCCGAGGCCCCGTAGCCCTCTGCCCTGCCCCCAGGTGGCCGCCACCC		
W V A E A L S R S A L L P P G G P P P P		
3670	3690	3710
CTGACCTGCCTGGGTCAAAGACTGAGCCCTGCTGGCGGACTTCAGGAGAACCCCCAC		
D L P G S K D *		
3730	3750	3770
AGGGGATTTGCTCCTAGAGTAAGGCTCATCTGGGCTCGGCCCGCACCTGGTGGCCT		
3790	3810	3830
TGTCCCTGAGGTGAGCCCCATGTCCATCTGGGCCACTGTCAAGGACCACTTGGGAGTGT		
3850	3870	3890
CATCCTTACAAACCAAGCATGCCCGCTCTCCAGAACCAAGTCCCAGCTGGGAGGAT		
3910	3930	3950
CAAGGCTGGATCCCCGGCGTTATCCATCTGGAGGCTGCAGGGTCTTGGGTAACAGG		
3970	3990	4010
GACCACAGACCCCTCACCACTCACAGATTCTCACACTGGGAAATAAGCCATTCAAGA		
4030		
GGAAAAAAAAAAAAAA		

MVVEKEQSWIPKIFKKKTCTTFTVDSTDGGTLCQCGRPRTABPAVAMEDAFGAAVVTWDSDAHTTEKPTDAYELDFTGAG  
 SNFLRLSRTDPAAVYSLVTRTWGFRAPNLVSVLGGSGGPVLTQWLQDLLRRGLVRAAQSTGAWIVTGLHTGIRHVGVA  
 QMASTGGTKVVMGVAPGVVNRDTLINPKGSFPARYRWGDPEDGVQFPDYNYSAFELVDDGTHGCLGGENRFRRLRLESY  
 QKTVGGGTGIDIPVLLLIDGDEKMLTRIENATQAHVPCLLVAGSRLGMPGGTLEAHIAQGDHKAQSTNQLLLPKDLSLC  
 S1DRKT1QSYSERLAVANRVDIAQSELFRGDIQWRSFHEASLMDALLNDRPEFVRLIISHLGLHFLTPMRLAQLYSAAI  
 L1RNLDDQASHSACTKAPALKGGAAELRPPDVGHVLRMLLGKMCAPRYPSSGAWDPHPGQGFESMYLSDKATSPSLDAGI  
 PWSDELLWALLLNRAQMAMYFWEMG3NAVSSALGACLLLRVMARLEPDAAEAAARRKDIAFKFEGMGVDLFGECEYRSSEVRAAF  
 RRCPLNGDATCLQLAMQADARAFFAQDGVSQSLLTQKWWGDMASTTP1IWALVLAFFCPPLIYTRLITFRKSEEETPREELEFDM  
 INGEGPVGTADEPAEKTPLGVPRQSGRPGCCGGRGRRCLRRWFHFWGVPTIFMGNVVSYLLFLLLFSRVLLVDFQPAPPGL  
 LLYFWAFTLLCEELRQGLSGGGGS1ASGGPGPGHASLSQRLLYLAQDSWNQCDLVALTCFLVGVCRLTPGLYHLGRTVLCII  
 FTVRLLH1FTVNVKQLGPKIVIVSKMMKDVFVFFLFLGVWLVAYGVATEGLLRPRDSDFPSILRRVFYRPyLQIFGOIPQEDMI  
 MEHSNCSESEPGFWAHPPGAQAGTCVSQYANWLVLVLLVIFLLVANILLVNLILIAMFSYTFGKVQGNSDLYWKAQRYRLTREFH  
 ALAPPFIVISHLRLLRLQLCRRPRSPQSPSSPALEHFRVYLSKEAERKLLTWEVHKENFLRARARDKRESERLKRTSQKVI  
 KQLGHIREYEQRILKVIEREVQQCSRVLGVVAEALSRSAALLPPGGPPPDLPGSKD

B.

10	30	50
ATCCAATGGCGGTCTTCCATCTCGAAGCTTCCCTCATGGACGCCCTGCTGAATGACCGG		
70	90	110
CCTGAGTTCGTGCCTTGCTCATTCCACGCCCTCAGCCTGGCCACTTCTGACCCCG		
130	150	170
ATGCGCTTGGCCCAACTCTACAGCGCGGCCCTCCAACTCGCTCATCCGCAACCTTTG		
190	210	230
GACCAGCGTCCCACAGCGCAGGCACCAAGGCCCTAAAGGGGGAGCTGCGGAG		
250	270	290
CTCCGGCCCTGACGTGGGCATGTGCTGAGGATGCTGCTGGGAAGATGTCGCCGCC		
310	330	350
AGATGTATCTGCTCTGGACAAGGCCACCTCGCCGCTCTCGCTGGATGCTGGCTCGGGC		
M Y L L S D K A T S P L S L D A G L G Q		
370	390	410
AGGCCCTGGAGCGACCTGCTTGGGACTGTGCTGAACAGGGCACAGATGGCA		
A P W S D L L W A L L L N R A Q M A M		
430	450	470
TGTACTCTGGGAGATGGGTTCAATGCAGTTCTCTGAGCTCTGGGCTGTTGCTGC		
Y F W E M G S N A V S S A L G A C L L L		

Fig. 9 / continuation 4

490	510	530
TCCGGGTGATGGCACGCCCTGGAGCCTGACGCTGAGGGAGGCAGCACGGAGGAAGACCTGG		
R V M A R L E P D A E E A A R R K D L A		
550	570	590
CGTTCAAGTTGAGGGGATGGCGTTGACCTCTTGGCGAGTGCTATCGCAGCAGTGAGG		
F K F E G M G V D L F G E C Y R S S E V		
610	630	650
TGAGGGCTGCCGCCCTCCCTCCGTCGCTGCCCGCTCTGGGGGATGCCACTTGCCCTCC		
R A A R L L L R R C P L W G D A T C L Q		
670	690	710
AGCTGGCCATGCAAGCTGACGCCGTGCCCTCTTGCCCAGGATGGGGTACAGTCCTGC		
L A M Q A D A R A F F A Q D G V Q S L L		
730	750	770
TGACACAGAAGTGGTGGGAGATATGCCAGCACTACACCCATCTGGGCCCTGGTCTCG		
T Q K W W G D M A S T T P I W A L V L A		
790	810	830
CCTCTTTGCCCTCCACTCATCACACCCGCCCTCATCACCTTCAGGAAATCAGAAGAGG		
F F C P P L I Y T R L I T F R K S E E E		
850	870	890
AGCCCACACGGGAGGAGCTAGAGTTGACATGGATAGTGTCAATTGGGAAGGGCCTG		
P T R E E L E F D M D S V I N G E G P V		
910	930	950
TCGGGACGGCGGACCCAGCCAGAAGACGCCGTGGGGTCCCGCCAGTCGGCCGTC		
G T A D P A E K T P L G V P R Q S G R P		
970	990	1010
CGGGTTGCTGGGGGGCGCTGGGGGGGGCGCCGGTGCCTACCCGCTGGTCCACTTCT		
G C C G G R C G G R R C L R R W F H F W		
1030	1050	1070
GGGGCGTGCCTGGTACCATCTCATGGGCAACGTGGTCAGCTACCTGCTGTCCTGCTGC		
G V P V T I F M G N V V S Y L L F L L L		
1090	1110	1130
TTTTCTCGCGGGTGCCTGCTGGATTCCAGCCGGCGCCGCCGGCTCCCTGGAGCTGC		
F S R V L L V D F Q P A P P G S L E L L		
1150	1170	1190
TGCTCTATTCTGGCTTACGCTGCTGCGAGGAACGTGCCAGGGCTGAGCGGAG		
L Y F W A F T L L C E E L R Q G L S G G		
1210	1230	1250
GCGGGGGCAGCCTGGCCAGCGGGGGCCCCGGGCTGGCATGCCACTGAGCCAGGCC		
G G S L A S G G P G P G H A S L S Q R L		
1270	1290	1310
TGCGCCCTACCTCGCCGACAGCTGGAACCGAGTGCACCTAGGGCTCTCACCTGCTTCC		
R L Y L A D S W N Q C D L V A L T C F L		
1330	1350	1370
TCCTGGCGTGGCTGCCGTGACCCCGGGTTGTACCACTGGCCGACTGTCTCT		
L G V G C R L T P G L Y H L G R T V L C		
1390	1410	1430
GCATCGACTCATGGTTTACGGTGGCGTGCCTCACATCTCACGGTCAACAAACAGC		
I D F M V F T V R L L H I F T V N K Q L		
1450	1470	1490
TGGGGCCCAAGATCGTACATCGTGAGCAAGATGATGAAGGACGTGTTCTTCTTCT		
G P K I V I V S K M M K D V F F F L F F		
1510	1530	1550
TCCTCGCGTGTGGCTGGTAGCCTATGGCGTGGCCACGGAGGGGCTCCTGAGGCCACGG		
L G V W L V A Y G V A T E G L L R P R D		
1570	1590	1610
ACAGTGACTTCCCAAGTATCCTGCCCGCTTCTACCGTCCCTACCTGCAGATCTCG		
S D F P S I L R R V F Y R P Y L Q I F G		
1630	1650	1670
GGCAGATTCCCCAGGAGGACATGGACGTGGCCCTCATGGAGCACAGCAACTGCTCGG		
Q I P Q E D M D V A L M E H S N C S S E		
1690	1710	1730
AGCCCGGCTCTGGCACACCTCCTGGGGCCAGGCCGGCACCTGCGTCTCCAGTATG		

Fig. 9 / continuation 5

P	G	F	W	A	H	P	P	G	A	Q	A	G	T	C	V	S	Q	Y	A													
1750									1770									1790														
CCA	ACT	GGC	TGG	TGG	TGG	TGT	GCT	CCT	CGT	CAT	CTT	CCT	GCT	CGT	GGC	AA	CAT	CCT	GCT	GG												
N	W	L	V	V	L	L	L	V	I	F	L	L	V	A	N	I	L	L	V													
1810									1830									1850														
TCA	ACT	GCT	CAT	TG	CC	CAT	GTT	CAG	TAC	AC	AT	CGG	AA	AGT	AC	AGG	GC	AA	AGC	GATC												
N	L	L	I	A	M	F	S	Y	T	F	G	K	V	Q	G	N	S	D	L													
1870									1890									1910														
TCT	ACT	GG	AAG	GG	CG	CAG	GCT	TAC	CC	GC	CT	AT	CC	GG	AA	TT	CC	AC	CT	CG	CC	CG	CT	GG								
Y	W	K	A	Q	R	Y	R	L	I	R	E	F	H	S	R	P	A	L	A													
1930									1950									1970														
CCCC	GCCCC	TTT	T	AT	CG	T	CAT	CT	CC	CA	T	TG	CG	CC	GC	AC	GG	CA	AT	TG	GC	AGG	CG	AC								
P	P	F	I	V	I	S	H	L	R	L	L	R	Q	L	C	R	R	P														
1990									2010									2030														
CCCCGGAGCCCC	CAG	CC	GT	CCT	CCC	GG	CC	CT	CG	AG	C	AT	TC	CG	GG	TT	AC	TT	CT	AA	GG	R	S	E								
R	S	P	Q	F	S	S	P	A	L	E	H	F	R	V	Y	L	S	K	E													
2050									2070									2090														
AAGCCGAGCGGAAG	CTG	CT	AA	CG	TG	GG	AA	TC	GG	TC	AT	AG	GA	AA	CT	TC	GT	GG	AC													
A	E	R	K	L	L	T	W	E	S	V	H	K	E	N	F	L	L	A	R													
2110									2130									2150														
GCG	CT	AGG	ACA	AG	CG	GG	AG	AG	CG	CA	T	CC	GG	CT	GA	AG	CG	AC	GT	CC	AG	AG	GT	GG								
A	R	D	K	R	E	S	D	S	E	R	L	K	R	T	S	Q	K	V	D													
2170									2190									2210														
ACTT	TGG	CA	T	AA	CA	AG	CT	GG	GA	CA	AC	AT	CC	GG	AG	T	AC	GA	AC	AG	CG	CC	TC	AA	AG	GT	CT	GG				
L	A	L	K	Q	L	G	H	I	R	E	Y	E	Q	R	L	K	V	L	E													
2230									2250									2270														
AGCGGGAGGT	CC	AG	CG	AG	TG	AG	CC	CG	TC	CT	GG	GG	GT	GG	GT	GG	CC	GC	AG	CC	CG	CT	AG	CC	CG	CT	GG					
R	E	V	Q	Q	C	S	R	V	L	G	W	V	A	E	A	L	S	R	S													
2290									2310									2330														
CTGC	CT	TG	CT	GC	CCCC	CAG	GT	GG	CC	GC	CC	CC	CT	GA	CT	GC	CT	GG	GT	CA	AA	AG	AC	GT	AG	CT	GG					
A	L	L	P	P	G	G	P	P	P	P	P	P	D	L	P	G	S	K	D	*												
2350									2370									2390														
CCCT	GT	TG	CG	GG	GA	CT	CA	AG	GA	GA	AG	CC	CC	CC	AC	AG	GG	GG	AT	TT	TG	CT	CT	AG	AG	GT	GG					
2410									2430									2450														
CAT	CT	GG	GC	CT	CG	GG	CC	CC	CC	GC	AC	CT	GG	GT	GG	CT	TC	CT	GG	GT	AG	CC	CC	AT	GT	CC	AT					
2470									2490									2510														
CTGGGCC	ACT	GT	TC	AG	GG	AC	AC	CT	TT	GG	AG	GT	TC	AT	CC	CT	TA	AA	AC	AC	AG	CAT	GC	CC	GG							
2530									2550									2570														
CTCC	CT	CC	CAG	AA	CC	AG	T	CC	AG	CC	TC	GG	AG	GT	CA	AG	GC	CT	GG	AT	CC	GG	CC	GT	T	AT	CC					
2590									2610									2630														
ATCTGGAGG	GT	CG	AG	GG	GT	CT	TT	GG	GG	AG	GT	TC	AT	CC	CT	TA	AA	CC	AC	AG	AC	CC	CT	AC	CA	CA	GA					
2650									2670									2690														
TTC	CT	CA	CA	CT	GG	GG	AA	AA	AA	AG	CC	AT	TC	AG	GG	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA			

MYLLSDKATSPLSLDAGLGQAPWSDLLWALLLNRAQMAMYFWEWGSNAVSSALGACLLLRLVMARLEPDAAERRDKLAFKEGM  
 GVDLFGECKRSSEVRAARLLRRCPLWGDAACLQLAMQADARAFFAQDGVSLLTQKWGDMASTTPIVALVIAFFCPPLIYTRLI  
 TFRKSEEEPTREELEFDMDSVINGEGPVGTADPAEKTPLGVPQRSGRPGCCGGRGRCGRCLRRWFHWGVPVTIFMGNVVSYLLFL  
 LLFSRVLIVDFQPAPPGLSLELLLYFWAFTLLCELRQGLSGGGSLASGGPGFHASLSQRRLRLYIADSWVNQCDLVALTCFLGVG  
 CRLTPGLYHLGRTVLCIDFMVFTVRLHIFTVNQKLGPKIVIVSKMMKDVFVFLFFLGWLVAYGVATEGLLPRSDFPSILRRV  
 FYRPYLQIFGQIPQEDMDVAlMEHSNCSEPGFWAHPPGQAQAGTCVSQYANLVVLLVIFLLVANILLVNLLIAMFSYTFGKVQG  
 NSDLYWKAQRYRLIREFHSRPALAPPFIVISHLRLRLQLCRRPRSPQPSSPALEHFRVYLSKEAERKLLTWEVHKENFLLRAR  
 DKRESDSERLKRTSQVKDLALKQ1GHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSLALLPPGGPPPPDLPGSKD

Fig. 10

A)

10	30	50
ATTAAAGTTATAAAACAGTGGCTGGATGGTTGGAGGATGCAGGTGGACAAAGACGTGG	M V G G C R W T E D V E	
70	90	110
AGCCTGCAGAAGTAAAGAAAAGATGCTCTTCGGGCAGCCAGGCTCAGCATGAGGAACA		
P A E V K E K M S F R A A R L S M R N R		
130	150	170
GAAGGAATGACACTCTGGACAGCACCCGGACCCCTGTACTCCAGCGCGTCTCGGAGCACAG		
R N D T L D S T R T I Y S S A S R S T D		
190	210	230
ACTTGTCCTACAGTGAAGCGCCAGCCTCTACGCTGCCCTCAGGACACAGACGTGCCAA		
L S Y S E S A S F Y A A F R T Q T C P I		
250	270	290
TCATGGCTCTGGGACTTGGTGAATTATTCAAGCAAATTAAAGAAACGAGAATGTG		
M A S W D L V N F I Q A N F K K R E C V		
310	330	350
TCTTCTTTACCAAAGATTCCAAGGCCACGGAGAACATGTGTGCAAGTGTGGCTATGCCAGA		
F F T K D S K A T E N V C K C G Y A Q S		
370	390	410
GCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAACCTACAAGAACACA		
Q H M E G T Q I N Q S E K W N Y K K H T		
430	450	470
CCAAGGAATTTCCTACCCACGCCCTTGGGATATTCAAGTGTGGGAAAGAAAG		
K E F P T D A F G D I Q F E T L G K K G		
490	510	530
GGAAGTATATACGTCTGCTCGCACCGGACGGCGGAAATCCTTACGAGCTGCTGACCC		
K Y I R L S C D T D A E I L Y E L L T Q		
550	570	590
AGCACTGGCACCTGAPAAACACCCAACCTGGTCAATTCTGTGACCGGGGGCGCCAAGAACT		
H W H L K T P N L V I S V T G G A K N F		
610	630	650
TCGCCCTGAAGCCGCGATGCGCAAGATCTCAGCCGGCTCATCTACATCGCGCAGTCCA		
A L K P R M R K I F S R L I Y I A Q S K		
670	690	710
AAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAGTACATCGGGAGG		
G A W I L T G G T H Y G L M K Y I G E V		
730	750	770
TGGTGAGAGATAACACCATCAGCAGGAGTCAGAGGAGAACATGTGGCCATTGGCATAG		
V R D N T I S R S S E E N I V A I G I A		
790	810	830
CAGCTTGGGCATGGCTCCAACCGGGACACCCCTCATCAGGAATTGCGATGCTGAGGGCT		
A W G M V S N R D T L I R N C D A E G Y		
850	870	890
ATTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTGTATATCCTGGACA		
F L A Q Y L M D D F T R D P L Y I L D N		
910	930	950
ACAACCCACACACATTGCTGCTCGGACAATGGCTGTATGGACATCCACTGTCGAAG		
N H T H L L L V D N G C H G H P T V E A		
970	990	1010
CAAAGCTCCGGAATCAGCTAGAGAAGTATATCTGTGAGCGCACTATTCAAGATTCCAAC		
K L R N Q L E K Y I S E R T I Q D S N Y		
1030	1050	1070
ATGGTGGCAAGATCCCCATTGTGTGTTTGCCTAAGGAGGTGGAAAGAGACTTGAAG		
G G K I P I V C F A Q G G G K E T L K A		
1090	1110	1130
CCATCAATACCTCCATCAAAAATAAAAATTCTGTGTGGTGGAGGCTCGGGCCAGA		
I N T S I K N K I P C V V V E G S G Q I		
1150	1170	1190
TCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACATCTCTGCCGTCA		
A D V I A S L V E V E D A L T S S A V K		
1210	1230	1250

Fig. 10 / continuation 1

AGGAGAAAGCTGGTGCCTTTTACCCGCACGGTGTCCCGCTGCCTGAGGAGGAGACTG  
 E K L V R F L P R T V S R L P E E E T E  
 1270 1290 1310  
 AGAGTTGGATCAAATGGCTAAAGAAATTCTCGAATGTTCTCACCTATTAACAGTTATTA  
 S W I K W L K E I L E C S H L L T V I K  
 1330 1350 1370  
 AAATGGAAGAAGCTGGGATGAAATTGTGAGCAATGCCATCTCCTACGCTATACAAAG  
 M E E A G D E I V S N A I S Y A L Y K A  
 1390 1410 1430  
 CCTTCAGCACCAAGTGAAGCAAGACAAGGATAACTGGAATGGCAGCTGAAGCTCTGCTGG  
 F S T S E Q D K D N W N G Q L K L L E  
 1450 1470 1490  
 AGTGGAACCGAGCTGGACTTAGCCAATGAGATTTCAACCAATGACCGCCGATGGAGA  
 W N Q L D L A N D E I F T N D R R W E K  
 1510 1530 1550  
 AGAGCAAACCGAGGCTCAGAGACACAATAATCCAGGTACATGGCTGGAAAATGGTAGAA  
 S K P R L R D T I I Q V T W L E N G R I  
 1570 1590 1610  
 TCAAGGTTGAGAGCAAAGATGTGACTGAGGCAAAGCCTCTTCATATGCTGGTGGTTC  
 K V E S K D V T D G K A S S H M L V V L  
 1630 1650 1670  
 TCAAGTCTGCTGACCTTCAGAAGTCATGTTACGGCTCTCATAAAGGACAGACCCAAGT  
 K S A D L Q E V M F T A L I K D R P K F  
 1690 1710 1730  
 TTGTCCGCCTTTCTGGAGAATGGCTTGACCTACCGAAGTTCTCACCCATGATGTCC  
 V R L F L E N G L N L R K F L T H D V L  
 1750 1770 1790  
 TCACTGAACCTTCTCCAACCACTTCAGCACGCTGTGTACCGGAATCTGCAGATGCCA  
 T E L F S N H F S T L V Y R N L Q I A K  
 1810 1830 1850  
 AGAATTCCATAATGATGCCCTCTCACGTTGCTGGAAACTGGTTGCGAACCTCCGAA  
 N S Y N D A L L T F V W K L V A N F R R  
 1870 1890 1910  
 GAGGCTTCGGAAGGAAGACAGAAATGGCGGGACGAGATGGACATAGAACTCCACGACG  
 G F R K E D R N G R D E M D I E L H D V  
 1930 1950 1970  
 TGTCTCCTATTACTCGGCACCCCTGCAAGCTCTTCATCTGGCCATTCTTCAGAATA  
 S P I T R H P L Q A L F I W A I L Q N K  
 1990 2010 2030  
 AGAAGGAACCTCCAAAGTCATTGGGAGCAGACCAGGGCTGCACTCTGGCAGCCCTGG  
 K E L S K V I W E Q T R G C T L A A L G  
 2050 2070 2090  
 GAGCCAGCAAGCTCTGAAGACTCTGGCAAAGTGAAGAACGACATCAATGCTGCTGGGG  
 A S K L L K T L A K V K N D I N A A G E  
 2110 2130 2150  
 AGTCCGAGGAGCTGGCTAATGAGTACGAGACCOGGCTGTTGGTGAGTCCACAGTGTGGA  
 S E E L A N E Y E T R A V G E S T V W N  
 2170 2190 2210  
 ATGCTGTGGTGGCGCGGATCTGCCATGTGGCACAGACATTGCCAGCGGCACTCATAGAC  
 A V V G A D L P C G T D I A S G T H R P  
 2230 2250 2270  
 CAGATGGTGGAGAGCTGTTCACTGAGTGTACAGCAGCGATGAAGACTGGCAGAACAGC  
 D G G E L F T E C Y S S D E D L A E Q L  
 2290 2310 2330  
 TGCTGGTCTATTCTGTGAAGCTGGGTGGAAGCAACTGCTCTGGAGCTGGCGGTGGAGG  
 L V Y S C E A W G G S N C L E L A V E A  
 2350 2370 2390  
 CCACAGACCAAGCATTCTCATGCCAGCCTGGGTCCAGAATTCTTCTTAAGCAATGGT  
 T D Q H F I A Q P G V Q N F L S K Q W Y  
 2410 2430 2450  
 ATGGAGAGATTCCCGAGACACCAAGAACGAACTGGAGATTATCCTGTGTGTTATTATAC  
 G E I S R D T K N W K I I L C L F I I P

Fig. 10 / continuation 2

2470	2490	2510
CCTTGGTGGGCTGTGGCTTGTATCATTTAGGAAGAAACCTGTCACAAGCACAGAAC		
L V G C G F V S F R K K P V D K H K K L		
2530	2550	2570
TGCTTTGGTACTATGTGGCGTCTTCACCTCCCCCTCGTGGCTTCTCTGGATGTGG		
L W Y Y V A F F T S P F V V F S W N V V		
2590	2610	2630
TCTTCTACATCGCCTTCCCTCGTGTGCTACGTGCTCATGGATTCCATTGG		
F Y I A F L L L F A Y V L L M D F H S V		
2650	2670	2690
TGCCACACCCCCCCCCGAGCTGGTCTGTACTCGCTGGTCTTGTCCCTCTGTGATGAAG		
P H P P E L V L Y S L V F V L F C D E V		
2710	2730	2750
TGAGACAGGGCCGGCCGGCTGCTCCAGTGCAGGGCCCGCAAGCCCCACGCCAACCGGA		
R Q G R P A A P S A G P A K P T P T R N		
2770	2790	2810
ACTCCATCTGGCCCGCAAGCTCCACACGCAGCCCCGGTCTCCGCTCACGCCACTCCTCC		
S I W P A S S T R S P G S R S R H S F H		
2830	2850	2870
ACACCTCCCTGCAAGCTGAGGGTGCCAGCTCTGGCCTTGGCCAGCCCAGAAAGGGGTGGA		
T S L Q A E G A S S G L G Q P R K G W T		
2890	2910	2930
CATTTAAAAATCTGGAAATGGTTGATATTTCCAAGCTGCTGATGTCCTCTCTGTCCCTT		
F K N L E M V D I S K L L M S L S V P F		
2950	2970	2990
TCTGTACGCAGTGGTACGTAAATGGGTGAATTATTTACTGACCTGTGGATGTGATGG		
C T Q W Y V N G V N Y F T D L W N V M D		
3010	3030	3050
ACACGCTGGGCTTTTACTTCATAGCAGGAATTGTATTCGGCAAGGGATCCTTAGGC		
T L G L F Y F I A G I V F R Q G I L R Q		
3070	3090	3110
AGAATGAGCAGCGCTGGAGGTGGATATTCCGTCGGTCATCTACGAGCCCTACCTGGCCA		
N E Q R W R W I F R S V I Y E P Y L A M		
3130	3150	3170
TGTTGGCCAGGTGCCAGTGACGTGGATGGTACCGTATGACTTGGCCACTGCACCT		
F G Q V P S D V D G T T Y D F A H C T F		
3190	3210	3230
TCACTGGGAATGAGTCCAAGCCACTGTGTGGAGCTGGATGAGCACAACCTGGGGT		
T G N E S K P L C V E L D E H N L P R F		
3250	3270	3290
TCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTGC		
P E W I T I P L V C I Y M L S T N I L L		
3310	3330	3350
TGGTCAACCTGCTGGTCGCCATGTTGGCTACACGGTGGCACCGTCCAGGAGAACATG		
V N L L V A M F G Y T V G T V Q E N N D		
3370	3390	3410
ACCAAGGTCTGGAAGTCCAGAGGTACTTCTGGTCAGGAGTACTGCAGCCCTCAATA		
Q V W K F Q R Y F L V Q E Y C S R L N I		
3430	3450	3470
TCCCCCTCCCTTCATCGTCTTCGTTACTTCTACATGGTGGTGAAGAACGTGCTCAAGT		
P F P F I V F A Y F Y M V V K K C F K C		
3490	3510	3530
GTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTGTGAGTGGTTATCCATGTGT		
C C K E K N M E S S V C C E W F I H V Y		
3550	3570	3590
ACTTGGGATCAGAAGCAGCGATTAATTCAAGGGAGGATGCCGCATCCAGTGAATTGGAA		
L G S E A A I N F R E G C L H P V I G S		
3610	3630	3650
GCTGGACCCAGGCTGGCTGGCTGGACATCCACACGCATTCTCACATGCAGTGCCGGCT		
W T P G W L V W T S T R I L T C S A G W		
3670	3690	3710
GGCCAGCAGCAGGGAGTCTCAGTGTACCCACACATAGCAGCTGGGTTCTGCAAAAGCA		

Fig. 10 / continuation 3

P	A	A	G	S	L	S	V	T	T	H	S	S	W	V	P	A	K	S	S
3730																			3770
GCAAGTCACAGGCCACCCAGACAGAACGGTAGAGAATGTGACTCTGCTCTGGTGGG																			
K	S	Q	A	H	P	D	R	T	G	R	E	C	D	S	A	S	G	W	E
3790																			3830
AAGGACAGCCTGCCGGTGGTGGAAAGAACCGTGGCCCTGTTGGCCATCGTGGCCCTG																			
G	Q	P	A	R	N	V	E	E	S	V	A	L	F	G	H	R	G	P	V
3850																			3890
TTGGCCACCTACCACTCTAGGCATCACTGAGCTGAATGCGCCGGTCCTCTGA																			
W	P	P	T	T	L	G	I	T	E	L	N	A	P	V	L	*			

MVGGCRWTEDVEPAEVKEKMSFRAARLSMRNRRNDTLDSTRTRLYSSASRSTDLSYSESASFYAAFRTQTCPIMASWDLVNFIQANF  
 KKRECVFFTKDSKATEVNCCKCGYAQSQHMEGTOINOSEKWNYKKHTKEFPTDAFGDIQFETLGKKGKYIIRLSCDTDAEILYELLTO  
 HHWLKTPNLVISVTGAKNFKLPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIIGEVVRDNTISRSSEENIVAGIAAWGMVS  
 NRDTLIRNCDAEYFLAQYLMDDFTRDPLYILDNNHHLVVNDNGCHGHPTVEAKLRNQLEKYIERTIQDSNYGGKIPIVCFAQG  
 GGKETLKANTSINKNIPCPVVVEGGSQIADVIASLVEVEDALTSSAVKEKLVRFLPRTVSRLPEEETESWIKWLKEILECSHLLTV  
 IKMEEAGDEIVSNASIYALYKAFSTSEQDKDNWNGQLKLLLEWNQLDLANDEIFTNDRRWBKSKPRLRTIIQVTWLENGRIKVES  
 KDVTDGKASSHMLVVLKSADLQEVMTALIKDRPKFVRLFLENGLNLRKFLLTDVLTFSNHFSTLVYRNLIQIAKNSYNDALLTF  
 VWKLVANFRRGFRKEDRNGRDEMIDIELHDVSPITRHPLQALFWAILQNKKELSKVIVEQTRGCTLAALGASKLILKTAKVKNDIN  
 AAGESEELANEYETRAVGESTVWNNAVVGADLPCGTDIASGTHRDPGGELFTECYSSDEDLAEQLLVYSCAEWGGSNCLELAVEATD  
 QHFIAQPGVQNFLSKQWYGEISRDTKNWKIIILCLFIIPLVGCGFVFRKKPVDKHKKLLMYYVAFFTSPPVVFSWNVVFYIAFLLL  
 FAYVLLMDFHSPVPHPELVLYSLVFLFCDEVRQGRPAAPSAGPAKPTPTRNSIWPASSSTRSPGSRSRHSFHTSLQAEGASSGLGQ  
 PRKGWTFKNLLEMVDISKLIMSLVPFCTQWYVNNGVNYFTDLNNMDTLLGLFYIFIAGIVFRQGILRQNQRWRWIFRSVIVYEPYLM  
 FGQVPSDVGTTYDFAHCTFTGNESKPLCVELDEHNLPRFPEWITIPLVCIYMLSTNILLVNLVAMFGYTVGTVQENNDOVWKFO  
 RYFLVQEYCSRLNIPFPFIVFAYFYMVVKCFCKKCKEKNMESSVCCCEWFIVYLGSEAAINFREGCLHPVIGSWTPGWLWWTSTR  
 ILTCSAGWPAAGSLSVTHSSWVPAKSSKSQAHPDRTGRECDASGWEQPARWVEESVALFGHRGPWPPTLGITELNAPVL

B.

Q L																			
2290	2310	2330																	
TGCTGGTCTATTCCCTGTGAAGCTGGGTGGAAGCAACTGTCCTGGAGCTGGCGGTGGAGG																			
L	V	Y	S	C	E	A	W	G	G	S	N	C	L	E	L	A	V	E	A
2350	2370	2390																	
CCACAGACCAGCATTTCATGCCAGCCTGGGTCCAGAACATTCTTCTAAAGCAATGGT																			
T	D	Q	H	F	I	A	Q	P	G	V	Q	N	F	L	S	K	Q	W	Y
2410	2430	2450																	
ATGGAGAGATTCCCGAGACACCAAGAACTGGAAGATTATCCTGTGTCTGTTATTATAC																			
G	E	I	S	R	D	T	K	N	W	K	I	I	L	C	L	F	I	I	P
2470	2490	2510																	
CCTTGGTGGCTGTGGCTTGTATCATTAGGAAGAACCTGTCGACAAGCACAAGAAGC																			
L	V	G	C	G	F	V	S	F	R	K	K	P	V	D	K				

Figure 11:

a.) Trp10b cDNA and derived amino acid sequence

10	30	50
ATGAAATCCTCCCTCCTGTCCACACCACATCGCTTATCAGGGAGAATGTGTGCAAGTGT		
M K S F L P V H T I V L I R E N V C K C		
70	90	110
GGCTATGCCAGAGCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAAC		
G Y A Q S Q H M E G T Q I N Q S E K W N		
130	150	170
TACAAAGAAACACACCAAGGAATTCCCTACCGACGCCCTGGGGATATTCAAGTTGAGACA		
Y K K H T K E F P T D A F G D I Q F E T		
190	210	230
CTGGGGAAAGAAAGGAAGTATATACGTCGTGCGACACGGACCGCGAAATCCTTAC		
L G K K G K Y I R L S C D T D A E I L Y		
250	270	290
GAGCTGCTGACCCAGCACTGGCACCTGAAAACACCCAACCTGGTCATTCTGTGACCGGG		
E L L T Q H W H L K T P N L V I S V T G		
310	330	350
GGGCCAAGAACCTCGCCCTGAAGCCCGCATGCGCAAGATCTTCAGCCGGCTCATCTAC		
G A K N F A L K P R M R K I F S R L I Y		
370	390	410
ATCGCGCAGTCACAAAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAG		
I A Q S K G A W I L T G G T H Y G L M K		
430	450	470
TACATGGGGAGGTGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTG		
Y I G E V V R D N T I S R S S E E N I V		
490	510	530
GCCATTGGCATAGCAGCTGGGCATGGCTCCAACCGGGACACCCCTCATCAGGAATTGC		
A I G I A A W G M V S N R D T L I R N C		
550	570	590
GATGCTGAGGGCTATTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTG		
D A E G Y F L A Q Y L M D D F T R D P L		
610	630	650
TATATCCTGGACAACAACCACACACATTGCTGCTCGTGGACAATGGCTGTATGGACAT		
Y I L D N N H T H L L L V D N G C H G H		
670	690	710
CCCACCTGCGAAGCAAAGCTCCGGAATCAGCTAGAGAAGTATATCTGAGCGCACTATT		
P T V E A K L R N Q L E K Y I S E R T I		
730	750	770
CAAGATTCCAACATATGGGGCAAGATCCCCATTGTGTGTTGCCAACGGAGGTGGAAAA		
Q D S N Y G G K I P I V C F A Q G G G K		
790	810	830
GAGACTTTGAAAGCCATCAATACCTCCATCAAAATAAAATTCCCTTGTTGGGGGGAA		
E T L K A I N T S I K N K I P C V V V E		
850	870	890
GGCTCGGGCCAGATCGCTGATGTGATCGCTAGCCTGGGGAGGTGGAGGATGCCCTGACA		
G S G Q I A D V I A S L V E V E D A L T		
910	930	950
TCTTCTGCCGTCAAGGAGAAGCTGGTGCCTTTTACCCCGCACGGTGTCCCGGCTGCCT		
S S A V K E K L V R F L P R T V S R L P		
970	990	1010
GAGGAGGAGACTGAGAGTTGGATCAAATGGCTCAAAGAAATTCTCGAAATGTTCTCACCTA		
E E E T E S W I K W L K E I L E C S H L		
1030	1050	1070
TTAACAGTTATAAAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTAC		
L T V I K M E E A G D E I V S N A I S Y		
1090	1110	1130
GCTCTATACAAAGCCTTCAGCACCACTGAGCAAGACAAGGATAACTGGAATGGGAGCTG		
A L Y K A F S T S E Q D K D N W N G Q L		

Fig. 11 (Continuation)

2410	2430	2450
AGAAACTTAGGACCCAGATTATAATGCCTGCAGAGGATGCTGATCGATGTGTTCTTCTTC		
R N L G P K I I M L Q R M L I D V F F F		
2470	2490	2510
CTGTTCCCTCTTGCGGTGTGGATGGTGGCCTTGGCGTGGCCAGGCAAGGGATCCTTAGG		
L F L F A V W M V A F G V A R Q G I L R		
2530	2550	2570
CAGAACGAGCGCTGGAGGTGGATATTCCGTTGGTCATCTACGAGCCCTACCTGGCC		
Q N E Q R W R W I F R S V I Y E P Y L A		
2590	2610	2630
ATGTTGGCCAGGTGCCAGTGACGTGGATGGTACCGTATGACTTTGCCACTGCACC		
M F G Q V P S D V D G T T Y D F A H C T		
2650	2670	2690
TTCACTGGGAATGAGTCCAAGCCACTGTGTGGAGCTGGATGAGCACAAACCTGCCCGG		
F T G N E S K P L C V E L D E H N L P R		
2710	2730	2750
TTCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCTG		
F P E W I T I P L V C I Y M L S T N I L		
2770	2790	2810
CTGGTCAACCTGGTCGCCATGTTGGCTACACGGTGGCACCGTCCAGGAGAACAT		
L V N L L V A M F G Y T V G T V Q E N N		
2830	2850	2870
GACCAGGTCTGGAAGTCCAGAGGTACTTCCCTGGTGCAGGAGTACTGCAGCCGCTCAAT		
D Q V W K F Q R Y F L V Q E Y C S R L N		
2890	2910	2930
ATCCCCCTCCCTTCATCGTCTCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAG		
I P F P F I V F A Y F Y M V V K K C F K		
2950	2970	2990
GTGGCTGCAAGGAGAAAAACATGGAGTCTCTGCTGTTCAAAATGAAGACAAT		
C C C K E K N M E S S V C C F K N E D N		
3010	3030	3050
GAGACTCTGGCATGGAGGGTGTCAAGGAAACTACCTGTCAAGATCAACACAAAA		
E T L A W E G V M K E N Y L V K I N T K		
3070	3090	3110
GCCAACGACACCTCAGAGGAAATGAGGCATGATTAGACAACCTGGATACAAAGCTTAAT		
A N D T S E E M R H R F R Q L D T K L N		
3130	3150	
GATCTCAAGGGTCACTGAAAGAGATTGCTAATAAAATCAAATAG		
D L K G L L K E I A N K I K *		

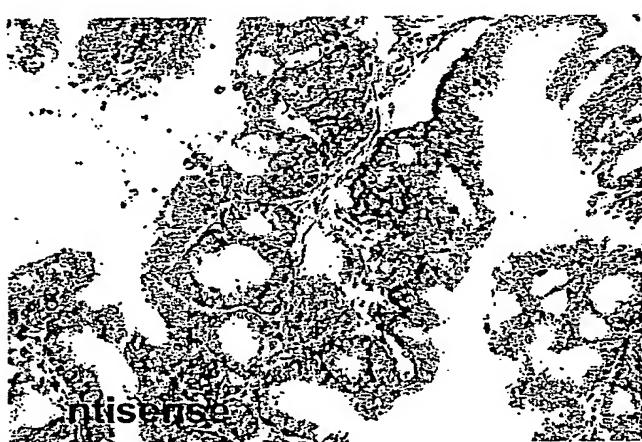
## b.) Trp10 protein:

MKSFLPVHTIVLIRENVCKCGYAQSQHMEGTQINQSEKWNKKHTKEFPTDAFGD1QFETLGKKKYIRLSCDTEAILEY  
 ELLTQHWHLKTPNLVISVTGGAKNFKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIV  
 AIGIAAWGMVSNRDTLIRNCDAEGLYFLAQYLMDDFTRDPLYILDNNHHTLLLVDNGCHGHTVEAKLRNQLEKYISERTI  
 QDSNYGGKIPIVCFAGQGGKETLKAINS1KNNK1PCVVEGSGQIADVIASLVEVEDALTSSAVERKEKLVRFPLRTVSRLP  
 EEEETESWIKWLKEILECSHLLTVIKMEEAGDEIVSNAISYALKAFTSEQDKDNWNGQLKLLLEWNQQLDLANDEIFTND  
 RRWESADLQEVMFTALIKDRPKFVRLFLENGLNLRKFLTHDVLTEFLFSNHFSTLVYRNQIAKNSYNDALLTFVWKLVAN  
 FRRGFRKEDRNGRDEMDEIHDVSPITRHPQLFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNINA  
 AGESEELANEYETRAVELFTECYSSDEDLAEOLLVYSCAEWGGSNCLELAVEATDQHFIAQPGVQNPLSKQWYGEISRTD  
 KNWKIIILCLFIIPLVGCGFVFSRKPKVDKHKKLLWYYVAFTSPFVVFWSNVVFYIAFLLLFAYVLLMDFHSVPHPPELV  
 LYSLVFVLFCDERVQWYVNGVNFTDLWNVMDTGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIFTLRLIHFVTS  
 RNLGPKIIIMLQRLMIDLVFFFLFLFAVWMVAFGVARQGILRQNEQRWRWIIFRSVIYEPYLMFGQVPSDVGTTYDFAHCT  
 FTGNESKPLCVELDEHNLPFPEWITIPLVCIYMLSTNILLVNLVAMFGYTVGTVQENNDQVWKFQRYFLVQEYCSRLN  
 IPFPFIVFAYFYMVVKCFKCCCKEKNMESSVCCFKNEDNETLAWEGVMKENYLVKINTKANDTSEEMRHRFRQLDTKLN  
 DLKGLLKEIANKIK

**The Trp8 Gene is expressed in normal endometrium**

**Trp8 mRNA in endometrial or uterine cancer**

**Endometrial cancer:**



**Endometrium:**



# Expression of human Trp 9 and Trp 10

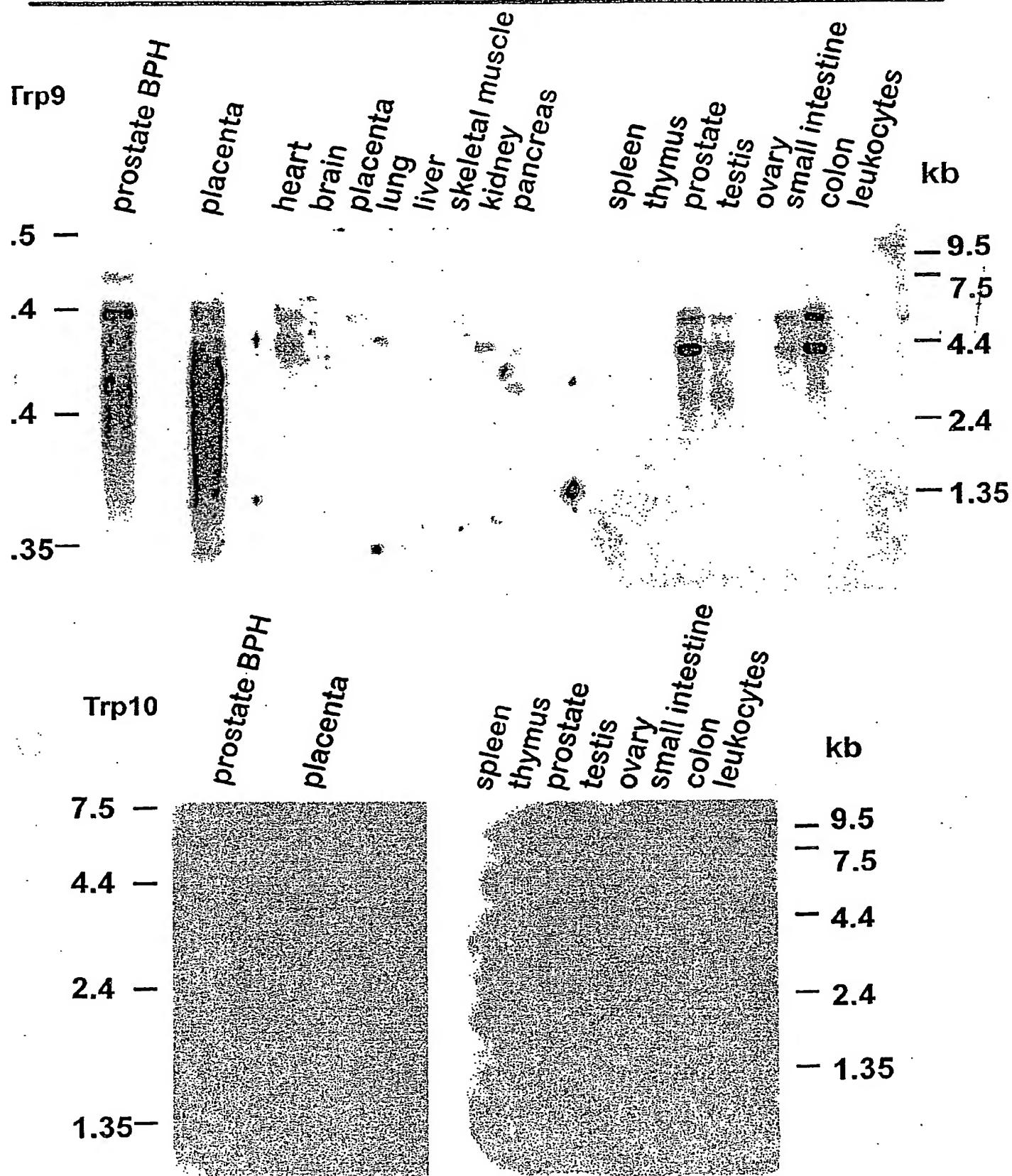
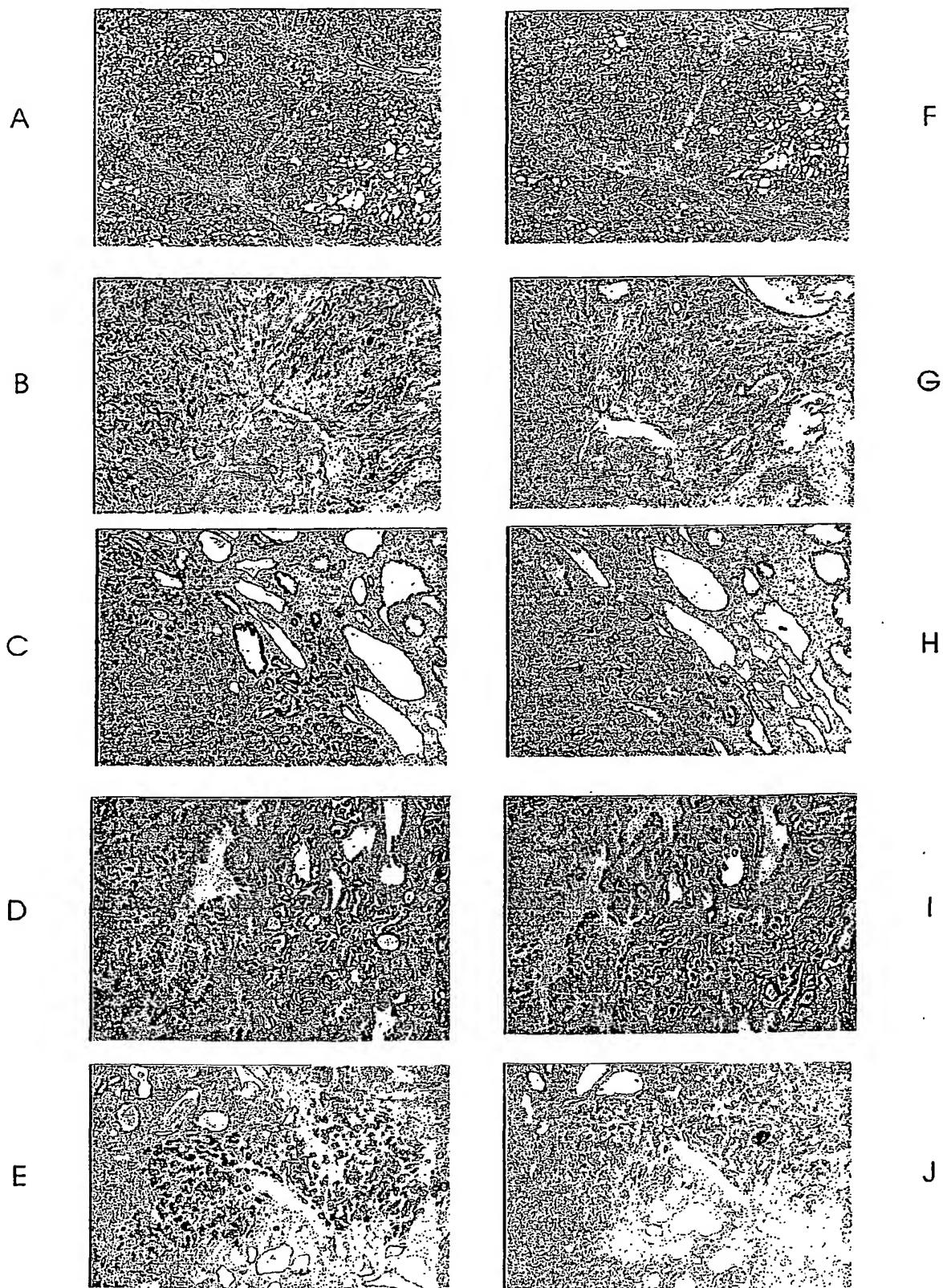


Fig. 14

Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and in malignant melanoma



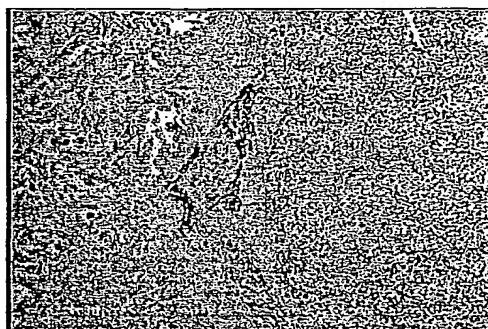
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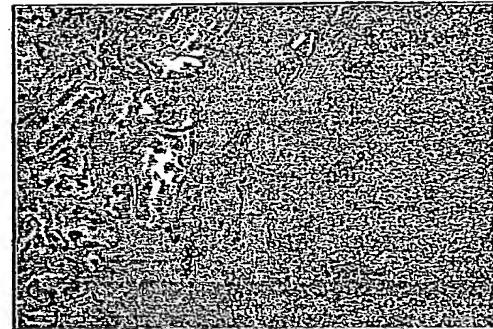
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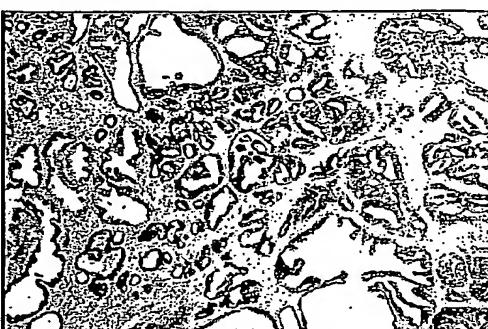
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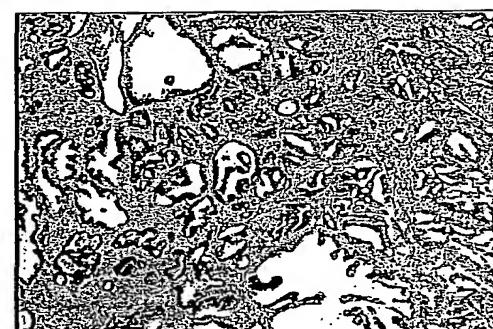
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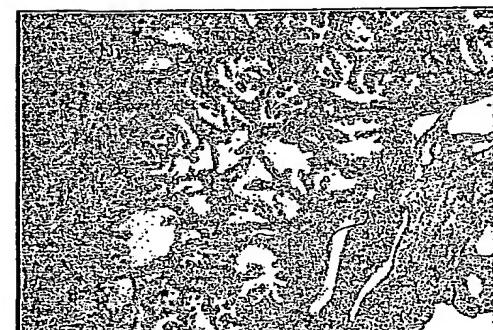
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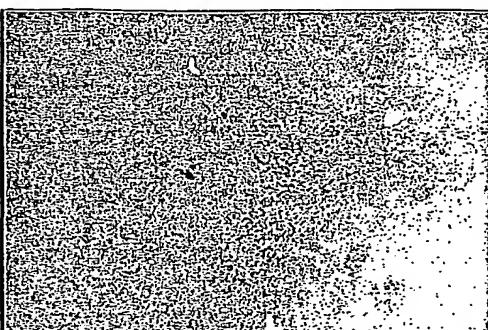
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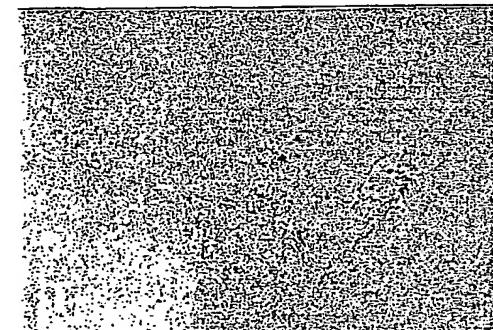
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(71) Applicant and

(72) Inventor: WISSENBACH, Ulrich [DE/DE]; Institut für Pharmakologie und Toxikologie der Uni, versität des Saarlandes, 66421 Homburg (DE).

(74) Agent: HUBER, Bernard; Huber &amp; Schüssler, Truderinger Str. 246, 81825 München (DE).

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(54) Title: TRP8, TRP9 AND TRP10, NOVEL MARKERS FOR CANCER

(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

**Trp8, Trp9 and Trp10, novel markers for cancer**FIELD OF THE INVENTION

The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10

BACKGROUND OF THE TECHNOLOGY

Prostate cancer is one of the most common diseases of older men world wide. Diagnosis and monitoring of prostate cancer is difficult because of the heterogeneity of the disease. For diagnosis different grades of malignancy can be distinguished according to the Gleason-Score Diagnosis. For this diagnosis a prostate tissue sample is taken from the patient by biopsy and the morphology of the tissue is investigated. However, this approach only yields subjective results depending on the experience of the pathologist. For confirmation of these results and for obtaining an early diagnosis an additional diagnostic method can be applied which is based on the detection of a prostate specific antigen (PSA). PSA is assayed in serum samples, blood samples etc. using an anti-PSA-antibody. However, since in principle PSA is also expressed in normal prostate tissue there is a requirement for the definition of a threshold value (about 4 ng/ml PSA) in order to be able to distinguish between normal and malign prostate tissue. Unfortunately, this diagnostic method is quite insensitive and often yields false-positive results. Moreover, by using this diagnostic method any conclusions as regards the grade of malignancy, the progression of the tumor and its potential for metastasizing cannot be drawn. Thus, the use of molecular markers would be helpful to distinguish benign from malign tissue and for grading and staging prostate carcinoma, particularly for patients with metastasizing prostate cancer having a very bad prognosis.

The above discussed limitations and failings of the prior art to provide meaningful specific markers which correlate with the presence of prostate tumors, in particular metastasizing tumors, has created a need for markers which can be used diagnostically, prognostically and therapeutically over the course of this disease. The present invention fulfills such a need by the provision of Trp8, Trp9 and Trp10 and the genes encoding Trp8, Trp9 and Trp10: The genes encoding Trp8 and Trp10 are expressed in prostate carcinoma and prostatic metastasis, but

not in normal prostate, benign hyperplasia (BHP) and intraepithelial prostatic neoplasia (PIN). Furthermore, expression of Trp10 transcripts is detectable in carcinoma but not in healthy tissue of the lung, the prostate, the placenta and in melanoma.

### SUMMARY OF THE INVENTION

The present invention is based on the isolation of genes encoding novel markers associated with cancer, Trp8, Trp9 and Trp10. The new calcium channel proteins Trp8, Trp9 and Trp10 are members of the trp (transient receptor potential) - family, isolated from human placenta (Trp8a and Trp8b) and human prostate (Trp9, Trp10a and Trp10b). Trp proteins belong to a steadily growing family of  $\text{Ca}^{2+}$  selective and non selective ion channels. In the recent years seven Trp proteins (trp1 - trp7) have been identified and suggested to be involved in cation entry, receptor operated calcium entry and pheromone sensory signaling. Structurally related to the trp proteins are the vanilloid receptor (VR1) and the vanilloid like receptor (VRL-1) both involved in nociception triggered by heat. Furthermore, two calcium permeable channels were identified in rat small intestine (CaT1) and rabbit kidney (ECaC). These distantly related channels are suggested to be involved in the uptake of calcium ions from the lumen of the small intestine (CaT1) or in the reuptake of calcium ions in the distal tubule of the kidney (ECaC). Common features of the Trp and related channels are a proposed structure comprising six transmembrane domains including several conserved amino acid motifs. In the present invention the cloning and expression of a CaT1 like calcium channel (Trp8) from human placenta as well as Trp9 and Trp10 (two variants, Trp10a and Trp10b) is described. Two polymorphic variants of the Trp8 cDNA were isolated from placenta (Trp8a and Trp8b). Transient expression of the Trp8b cDNA in HEK (human embryonic kidney) cells results in cytosolic calcium overload implicating that the Trp8 channel is constitutive open in the expression system. Trp8 induces highly calcium selective inward currents in HEK cells. The C-terminus of the Trp8 protein binds calmodulin in a calcium dependent manner. The Trp9 channel is expressed in trophoblasts and syncytiotrophoblasts of placenta and in pancreatic acinar cells. Furthermore, the Trp8 channel is expressed in prostatic carcinoma and prostatic metastases, but not in normal tissue of the prostate. No expression of Trp8 transcripts is detectable in benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN). Therefore, the Trp8 channel is exclusively expressed in malign prostatic tissues and serves as molecular marker for prostate cancer. From the experimental results it is also apparent that the

modulation of Trp8 and/or Trp10, e.g. the inhibition of expression or activity, is of therapeutic interest, e.g. for the prevention of tumor progression.

The present invention, thus, provides a Trp8, Trp9 and Trp10 protein, respectively, as well as nucleic acid molecule encoding the protein and, moreover, an antisense RNA, a ribozyme and an inhibitor, which allow to inhibit the expression or the activity of Trp8, Trp9 and/or Trp10.

In one embodiment, the present invention provides a diagnostic method for detecting a prostate cancer or endometrial cancer (cancer of the uterus) associated with Trp8 or Trp10 in a tissue of a subject, comprising contacting a sample containing Trp8 and/or Trp10 encoding mRNA with a reagent which detects Trp8 and/or Trp10 or the corresponding mRNA.

In a further embodiment, the present invention provides a diagnostic method for detecting a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense transcripts or Trp10a and/or Trp10b related antisense transcripts.

In another embodiment, the present invention provides a method of treating a prostate tumor, carcinoma of the lung, carcinoma of the placenta (chorion carcinoma) or melanoma associated with Trp8 and/or Trp10, comprising administering to a subject with such an disorder a therapeutically effect amount of a reagent which modulates, e.g. inhibits, expression of Trp8 and/or Trp10 or the activity of the protein, e.g. the above described compounds.

Finally, the present invention provides a method of gene therapy comprising introducing into cells of a subject an expression vector comprising a nucleotide sequence encoding the above mentioned antisense RNA or ribozyme, in operable linkage with a promoter.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A, phylogenetic relationship of trp and related proteins. B, hydropathy plot of the Trp8 protein sequence according to Kyte and Doolittle. C, alignment of Trp8a/b to the epithelial calcium channels ECaC (from rabbit) and Vr1 (from rat). Putative transmembrane domains are underlined.

**Figure 2:** A, polymorphism of the Trp8 gene. The polymorphic variants Trp8a and Trp8b differ in five base pairs resulting in three amino acid exchanges in the derived protein sequences. Specific primers were derived from the Trp8 gene as indicated by arrows. B, the Trp8a and Trp8b genes are distinguishable by a single restriction site. Genomic fragments of the Trp8 gene can be amplified using specific primers (shown in A). The genomic fragment of the Trp8b gene contains an additional site of the restriction enzyme BSP1286I (B). C, the Trp8 gene is located on chromosome 7. D, genotyping of eleven human subjects. A 458 bp genomic fragment of the Trp8 gene was amplified using specific primers (shown in A) and restricted with BSP1286I. The resulting fragments were analyzed by PAGE electrophoresis.

**Figure 3:** The Trp8b protein is a calcium selective ion channel. A, representative trace of a pdiTrp8b transfected HEK 293 cell. Trp8b mediated currents are activated by voltage ramps (-100 mV - +100 mV) of 100 msec at -40 mV or +70 mV holding potential. 1, Trp8b currents in the presence at 2 mM  $[Ca^{2+}]_o$ ; 2, effect of solution switch alone 3, switch to nominal zero calcium solution. B, Trp8b currents in the presence of zero divalent cations. C, current voltage relationship of the currents shown in A. Inset, leak subtracted current. D, current voltage relationship of the current shown in B. E, statistics of representative experiments. Black: Trp8 transfected cells, gray: control cells. Columns from left to right: Trp8 currents at -40 mV (n = 12) and +70 mV holding potential (n = 12). Trp8 currents in standard bath solution including 120 mM NMDG without sodium (n = 7) and with nominal zero calcium ions (n = 8) or in the presence of 1 mM EGTA with zero divalent cations (n = 6). F, representative changes in  $[Ca^{2+}]_i$  in Trp8b transfected HEK cells (gray) and controls (black) in the presence or absence of 1 mM  $[Ca^{2+}]_o$ . Inset, relative increase of cytosolic calcium concentration of Trp8b transfected HEK cells, before and after readdition of 1 mM  $[Ca^{2+}]_o$  in comparison to control cells.

**Figure 4:** The C-terminal region of the Trp8 protein binds calmodulin. A, N- and C-terminal fragments of the Trp8 protein used for calmodulin binding studies. B, the Trp8 protein and a truncated Trp8 protein which was in vitro translated after MunI cut of the cDNA, which lacks the C-terminal 32 amino acid residues, were in vitro translated in the presence of  $^{35}S$ -methionine and incubated with calmodulin coupled agarose beads in the presence of 1 mM  $Ca^{2+}$  or 2 mM EGTA. C, calmodulin binding to N- and C-terminal fragments of the Trp8 protein in the presence of  $Ca^{2+}$  (1 mM) or EGTA (2 mM)

Figure 5: Expression pattern of the Trp8 cDNA. A, Northern blots (left panels, Clontech, Palo Alto) were hybridized using a 348 bp NcoI/BamHT fragment of the Trp9 cDNA. The probe hybridizes to mRNA species isolated from the commercial blot, but not to mRNA species isolated from benign prostate hyperplasia (right panel, mRNA isolated from 20 human subjects with benign prostate hyperplasia). B,C, in situ hybridization with biotinylated Trp8 specific oligonucleotides on slides of human tissues. Left column antisense probes, right column sense probes. D, antisense probes.

Figure 6: Differential expression of Trp8 cDNA in human prostate. A - F, in situ hybridization with prostatic tissues. A, normal prostate, B, primary carcinoma, C, benign hyperplasia, D, rezidive carcinoma, E, prostatic intraepithelial neoplasia, F, lymphnode metastasis of the prostate.

Figure 7: Trp8a cDNA sequence and derived amino acid sequence

Figure 8: A, Trp8b cDNA sequence and derived amino acid sequence

B, cDNA sequence of splice variant 1 (12B1)

C, cDNA sequence of splice variant 2 (17-3)

D, cDNA sequence of splice variant 3 (23A3)

E, cDNA sequence of splice variant 4 (23C3)

Figure 9: A, Trp9 cDNA sequence and derived amino acid sequence B, cDNA sequence of splice variant 15 and derived amino acid sequence.

Figure 10: A, cDNA sequence of Trp10a and derived amino acid sequence, B, cDNA fragment of Trp10a and derived amino acid sequence.

Figure 11: cDNA sequence of Trp10b and derived amino acid sequence.

Figure 12: Expression of Trp8 mRNA in human endometrial cancer or cancer of the uterus. A - D, in situ hybridization with slides of endometrial cancer hybridized with Trp8 antisense (left column) or sense probes as controls (right column). E - F, Trp8 antisense probes hybridized to slides of normal endometrium. It can be clearly seen no hybridization occurs with normal endometrial tissue.

**Figure 13: Expression of human Trp9 and Trp10 genes**

Northern blots were hybridized using Trp9 (upper panel) or Trp10 (lower panel) specific probes. Expression of the Trp9 cDNA is detectable in many tissues including human prostate and colon as well as in benign prostatic hyperplasia. Expression of Trp10 cDNA is detectable in human prostate of a commercial northern blot (Clontech, right side). This Northern blot contains prostatic tissue collected from 15 human subjects in the range of 14 - 60 years of age. No expression of Trp10 cDNA was detectable in benign prostatic hyperplasia (left side).

**Figure 14: Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and metastasis of a melanoma.** In situ hybridizations of slides hybridized with Trp10-antisense (A-E, K-N) and Trp10 related sense probes (F-J, P-R). It can clearly be seen that both probes detect the same cancer cells indicating that these cancer cells express Trp10 transcripts as well as Trp10-antisense transcripts. S, no Trp10 expression is detectable in benign hyperplasia of the prostate (BPH). O and T, show expression of Trp10 transcripts (O) and Trp10-antisense transcripts (T) in a metastasis of a melanoma in human lung. Melanoma cancer cells express both Trp10 transcripts and Trp10-antisense transcripts.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

- (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11;
- (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10, or 11;
- (c) a nucleic acid molecule included in DSMZ Deposit no. DSM 13579 (deposit date: 28 June 2000), DSM 13580 (deposit date: 28 June 2000), DSM 13584 (deposit date: 5 July 2000), DSM 13581 (deposit date: 28 June 2000) or DSM ....(deposit date:....);
- (d) a nucleic acid molecule with hybridizes to a nucleic acid molecule specified in (a) to (c)

- (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
- (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

As used herein, a protein exhibiting biological properties of Trp8a, Trp8b, Trp9,Trp10a or Trp10b is understood to be a protein having at least one of the activities as illustrated in the Examples, below.

As used herein, the term „isolated nucleic acid molecule,“ includes nucleic acid molecules substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated.

In a first embodiment, the invention provides an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b comprising the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11. The present invention also provides a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10 or 11.

The present invention provides not only the generated nucleotide sequence identified in Figure 7, 8A, 9,10 or 11, respectively and the predicted translated amino acid sequence, respectively, but also plasmid DNA containing a Trp8a cDNA deposited with the DSMZ, under DSM 13579, a Trp8b cDNA deposited with the DSMZ, under DSM 13580, a Trp9 cDNA deposited with the DSMZ, under DSM 13584, a Trp10a cDNA deposited with the DSMZ, under DSM 13581, and a Trp10b cDNA deposited with the DSMZ, under DSM...., respectively. The nucleotide sequence of each deposited Trp-clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by each deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited Trp-encoding DNA, collecting the protein, and determining its sequence.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. It is understood that all

nucleic acid molecules encoding all or a portion of Trp8a, Trp8b, Trp9, Trp10a or Trp10b are also included, as long as they encode a polypeptide with biological activity. The nucleic acid molecules of the invention can be isolated from natural sources or can be synthesized according to known methods.

The present invention also provides nucleic acid molecules which hybridize to the above nucleic acid molecules. As used herein, the term „hybridize,“ has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual 2<sup>nd</sup> edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Also contemplated are nucleic acid molecules that hybridize to the Trp nucleic acid molecules at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from human cell lines or tissues. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., *supra*). As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic

fragments that were produced by means of conventional synthetic methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The nucleic acid molecules of the present invention also include molecules with sequences that are degenerate as a result of the genetic code.

In a further embodiment, the present invention provides nucleic acid molecules which comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. „Fragments,“ are understood to be parts of the nucleic acid molecules that are long enough to encode one of the described proteins. These fragments comprise nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules of the invention. These nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assay and/or kit described below and, preferably, are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can also be used, for example, as primers for a PCR reaction. Examples of particular useful probes (primers) are shown in Tables 1 and 2.

Table 1

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

1. ) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTTGAGGAT 3'

Tabelle 2

Trp10 probes used for the in situ hybridizations shown in Figure 14:

## Probes (antisense)

- 1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (Figure 14 A, 14B)
- 2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (Figure 14C, 14D, 14N, 14S, 14O)
- 3.) 5' ATCTTCCAGTTCTGGTGTCTCGG 3' (Figure 14E, 14K)
- 4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (Figure 14L, 14M)

## Probes (sense)

- 1.) 5' TCTATTCTGTGAAGCTTGGGTGGAAGC 3' (Figure 14F, 14G)
- 2.) 5' GCCACAGACCAGCATTTCATCGCC 3' (Figure 14H, 14I, 14T)
- 3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (Figure 14J, 14P)
- 4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (Figure 14Q, 14R)

The term „derivative,“ in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and particularly preferred of more than 90%. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in Figure 7, 8A, 9, 10 and 11, respectively, of at least 80%, preferably of 85% and particularly preferred of more than 90%, 97% and 99%. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination. The definition of the derivatives also includes splice variants, e.g. the splice variants shown in Figures 8B to 8E and 9B.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes.

Generally, by means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., *supra*) to introduce different mutations into the nucleic acid molecules of the invention. As a result Trp proteins or Trp related proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. Another possibility is the introduction of single-point mutation at positions where a modification of the amino acid sequence influences, e.g., the ion channel properties or the regulations of the trp-ion channel. By this method muteins can be produced, for example, that possess a modified ion conducting pore, a modified  $K_m$ -value or that are no longer subject to the regulation mechanisms that normally exist in the cell, e.g. with regard to allosteric regulation or covalent modification. Such muteins might also be valuable as therapeutically useful antagonists of Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., *supra*) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, in vitro mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as ion channel activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretical mobility, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability; pH optimum, temperature optimum.

The invention furthermore relates to vectors containing the nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors

usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the recombinant vector of the invention that guarantee the transcription and synthesis of an RNA in prokaryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter.

In a further embodiment, the present invention relates to recombinant host cells transiently or stably containing the nucleic acid molecules or vectors of the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention. Preferably, these cells are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The host cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to isolated proteins exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby, e.g., a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced proteins may be carried out by conventional means including preparative chromatography and affinity and immunological separations involving affinity with an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a- or anti-Trp10b-antibody, respectively.

As used herein, the term „isolated protein,“ includes proteins substantially free of other proteins, nucleic acids, lipids, carbohydrates or other materials with which it is naturally associated. Such proteins however not only comprise recombinantly produced proteins but include isolated naturally occurring proteins, synthetically produced proteins, or proteins

produced by a combination of these methods. Means for preparing such proteins are well understood in the art. The Trp proteins are preferably in a substantially purified form. A recombinantly produced version of a human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b protein, including the secreted protein, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67; 31-40 (1988).

In a further preferred embodiment, the present invention relates to an antisense RNA sequence characterised that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to said mRNA, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecules, and a ribozyme characterised in that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to and cleave said mRNA, thus inhibiting the synthesis of the proteins encoded by said nucleic acid molecules. Ribozymes which are composed of a single RNA chain are RNA enzymes, i.e. catalytic RNAs, which can intermolecularly cleave a target RNA, for example the mRNA transcribed from one of the Trp genes. It is now possible to construct ribozymes which are able to cleave the target RNA at a specific site by following the strategies described in the literature. (see, e.g., Tanner et al., in: *Antisense Research and Applications*, CRC Press Inc. (1993), 415-426). The two main requirements for such ribozymes are the catalytic domain and regions which are complementary to the target RNA and which allow them to bind to its substrate, which is a prerequisite for cleavage. Said complementary sequences, i.e., the antisense RNA or ribozyme, are useful for repression of Trp8a-, Trp8b, Trp9-, Trp10a- and Trp10b-expression, respectively, i.e. in the case of the treatment of a prostate cancer or endometrial cancer (carcinoma of the uterus). Preferably, the antisense RNA and ribozyme of the invention are complementary to the coding region. The person skilled in the art provided with the sequences of the nucleic acid molecules of the present invention will be in a position to produce and utilise the above described antisense RNAs or ribozymes. The region of the antisense RNA and ribozyme, respectively, which shows complementarity to the mRNA transcribed from the nucleic acid molecules of the present invention preferably has a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides.

In still a further embodiment, the present invention relates to inhibitors of Trp8a, Trp8b, Trp9, Trp10a and Trp10b, respectively, which fulfill a similar purpose as the antisense RNAs or

ribozymes mentioned above, i.e. reduction or elimination of biologically active Trp8a, Trp8b, Trp9, Trp10a or Trp10b molecules. Such inhibitors can be, for instance, structural analogues of the corresponding protein that act as antagonists. In addition, such inhibitors comprise molecules identified by the use of the recombinantly produced proteins, e.g. the recombinantly produced protein can be used to screen for and identify inhibitors, for example, by exploiting the capability of potential inhibitors to bind to the protein under appropriate conditions. The inhibitors can, for example, be identified by preparing a test mixture wherein the inhibitor candidate is incubated with Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively, under appropriate conditions that allow Trp8a, Trp8b, Trp9, Trp10a or Trp10b to be in a native conformation. Such an in vitro test system can be established according to methods well known in the art. Inhibitors can be identified, for example, by first screening for either synthetic or naturally occurring molecules that bind to the recombinantly produced Trp protein and then, in a second step, by testing those selected molecules in cellular assays for inhibition of the Trp protein, as reflected by inhibition of at least one of the biological activities as described in the examples, below. Such screening for molecules that bind Trp8a, Trp8b, Trp9, Trp10a or Trp10b could easily be performed on a large scale, e.g. by screening candidate molecules from libraries of synthetic and/or natural molecules. Such an inhibitor is, e.g., a synthetic organic chemical, a natural fermentation product, a substance extracted from a microorganism, plant or animal, or a peptide. Additional examples of inhibitors are specific antibodies, preferably monoclonal antibodies. Moreover, the nucleic sequences of the invention and the encoded proteins can be used to identify further factors involved in tumor development and progression. In this context it should be emphasized that the modulation of the calcium channel of a member of the trp family can result in the stimulation of the immune response of T lymphocytes leading to proliferation of the T lymphocytes. The proteins of the invention can, e.g., be used to identify further (unrelated) proteins which are associated with the tumor using screening methods based on protein/protein interactions, e.g. the two-hybrid-system Fields, S. and Song, O. (1989) Nature (340): 245-246.

The present invention also provides a method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.

It has been found that carcinoma cells of placenta (chorion carcinoma), lung and prostate express Trp10 transcripts as well as Trp10 antisense transcripts and transcripts being in part complementary to Trp10 antisense transcripts. Accordingly, the present invention also provides a method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA.

When the target is mRNA (or antisense RNA), the reagent is typically a nucleic acid probe or a primer for PCR. The person skilled in the art is in a position to design suitable nucleic acids probes based on the information as regards the nucleotide sequence of Trp8a, Trp8b, Trp10a or Trp10b as depicted in figure 7, 8a, 10 and 11, respectively, or tables 1 and 2, above. When the target is the protein, the reagent is typically an antibody probe. The term „antibody“, preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing fragments of the proteins of the invention by methods well known to those skilled in the art (see, e.g., Köhler et al., *Nature* **256** (1975), 495). As used herein, the term „antibody“ (Ab) or „monoclonal antibody“ (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and f(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., *J. Nucl. Med.* **24**: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies. The target cellular component, i.e. Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, e.g., in biological fluids or tissues, may be detected directly *in situ*, e.g. by *in situ* hybridization (e.g., according to the examples, below) or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern blot analysis, RNase protection, *in situ* methods, e.g. *in situ* hybridization, *in vitro* amplification methods (PCR, LCR, QRNA replicase or RNA-transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362)), immunoassays, Western blot and other detection assays that are known to those skilled in the art.

Products obtained by in vitro amplification can be detected according to established methods, e.g. by separating the products on agarose gels and by subsequent staining with ethidium bromide. Alternatively, the amplified products can be detected by using labeled primers for amplification or labeled dNTPs.

The probes can be detectable labeled, for example, with a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

Expression of Trp8a, Trp8b, Trp10a and Trp10b, respectively, in tissues can be studied with classical immunohistological methods (Jalkanen et al., *J. Cell. Biol.* **101** (1985), 976-985; Jalkanen et al., *J. Cell. Biol.* **105** (1987), 3087-3096; Sobol et al. *Clin. Immunopathol.* **24** (1982), 139-144; Sobol et al., *Cancer* **65** (1985), 2005-2010). Other antibody based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (<sup>125</sup>I, <sup>121</sup>I), carbon (<sup>14</sup>C), sulfur (<sup>35</sup>S), tritium (<sup>3</sup>H), indium (<sup>112</sup>In), and technetium rhodamine, and biotin. In addition to assaying Trp8a, Trp8b, Trp 10a or Trp10b levels in a biological sample, the protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, <sup>131</sup>I, <sup>112</sup>In, <sup>99</sup>mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99</sup>mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In

vivo tumor imaging is described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments“. (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

The marker Trp8a and Trp8b is also useful for prognosis, for monitoring the progression of the tumor and the diagnostic evaluation of the degree of malignancy of a prostate tumor (grading and staging), e.g. by using *in situ* hybridization: In a primary carcinoma Trp8 is expressed in about 2 to 10% of carcinoma cells, in a rezidive carcinoma in about 10 to 60% of cells and in metastases in about 60 to 90% of cells.

The present invention also relates to a method for diagnosing endometrial cancer (cancer of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the encoding mRNA and detecting Trp8a and/or Trp8b encoding mRNA. As regards particular embodiments of this method reference is made to the particular embodiments of the method of diagnosing a prostate cancer outlined above.

For evaluating whether the concentration of Trp8a, Trp8b, Trp10a or Trp10b or the concentration of Trp8a, Trp8b, Trp10a or Trp10b encoding mRNA is normal or increased, thus indicative for the presence of a malignant tumor, the measured concentration is compared with the concentration in a normal tissue, preferably by using the ratio of Trp8a:Trp9, Trp8b:Trp9 or Trp10(a or b)/Trp9 for quantification.

Since the prostate carcinoma forms its own basement membrane when growing invasively, it can be concluded that only cells expressing Trp8 and Trp10 are involved in this phenomenon. Thus, it can be concluded that by inhibiting the expression and/or activity of these proteins an effective therapy of cancers like PCA is provided.

Thus, the present invention also relates to a pharmaceutical composition containing a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b, and a method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (uterine carcinoma) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a

therapeutically effective amount of a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b. Examples of such reagents are the above described antisense RNAs, ribozymes or inhibitors, e.g. specific antibodies. Furthermore, peptides, which inhibit or modulate the biological function of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b may be useful as therapeutical reagents. For example, these peptides can be obtained by screening combinatorial phage display libraries (Cosmix, Braunschweig, Germany) as described by Rottgen, P. and Collins, J. (Gene (1995) 164 (2): 243-250). Furthermore, antigenic epitopes of the Trp8 and Trp10 proteins can be identified by the expression of recombinant Trp8 and Trp10 epitope libraries in *E. coli* (Marquart, A. & Flockerzi, V., FEBS Lett. 407 (1997), 137-140; Trost, C., et al., FEBS Lett. 451 (1999) 257-263 and the consecutive screening of these libraries with serum of patients with cancer of the prostate or of the endometrium. Those Trp8 and Trp10 epitopes which are immunogenic and which lead to the formation of antibodies in the serum of the patients can be then be used as Trp8 or Trp10 derived peptide vaccines for immune inventions against cancer cells which express Trp8 or Trp10. Alternatively to the *E. coli* expression system, Trp8 or Trp10 or epitopes of Trp8 and Trp10 can be expressed in mammalian cell lines such as human embryonic kidney (Hek 293) cells (American Type Culture Collection, ATCC CRL 1573).

Finally, compounds useful for therapy of the above described diseases comprise compounds which act as antagonists or agonists on the ion channels Trp8, Trp9 and Trp10. It could be shown that Trp8 is a highly calcium selective ion channel which in the presence of monovalent (namely sodium) and divalent ions (namely calcium) is only permeable for calcium ions (see Example 4, below, and Figures 3A, C, E). Under physiological conditions, Trp8 is a calcium selective channel exhibiting large inward currents. This very large conductance of Trp8 channels (as well as Trp9 and Trp10a/b channels) is useful to establish systems for screening pharmacological compounds interacting with Trp-channels including high throughput screening systems. Useful high throughput screening systems are well known to the person skilled in the art and include, e.g., the use of cell lines stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and Trp10 channels in assays to detect calcium signaling in biological systems. Such systems include assays based on Ca-sensitive dyes such as aequorin, apoaequorin, Fura-2, Fluo-3 and Indo-1.

Accordingly, the present invention also relates to a method for identifying compounds which act as agonists or antagonists on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, preferably by using a system based on cells stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

For administration the above described reagents are preferably combined with suitable pharmaceutical carriers. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the tumor and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the tumor, general health and other drugs being administered concurrently.

The delivery of the antisense RNAs or ribozymes of the invention can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. By delivering these nucleic acids to the desired target, the intracellular expression of Trp8a, Trp8b, Trp10a and/or Trp10b and, thus, the level of Trp8a, Trp8b, Trp10a and/or Trp10b can be decreased resulting in the inhibition of the negative effects of Trp8a, Trp8b, Trp10a and/or Trp10b, e.g. as regards the metastasis formation of PCA.

Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic acids include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions

(mixed), micelles, liposomes and lipoplexes. The preferred colloidal system is a liposome. The composition of the liposome is usually a combination of phospholipids and steroids, especially cholesterol. The skilled person is in a position to select such liposomes which are suitable for the delivery of the desired nucleic acid molecule. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tumor. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tumors via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, i.e. tumor to be treated, the nucleic acids encoding, e.g. an antisense RNA or ribozyme can also be operably linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) *Neuron* 12, 11-24; Vidal et al.; (1990) *EMBO J.* 9, 833-840; Mayford et al., (1995), *Cell* 81, 891-904; Pinkert et al., (1987) *Genes & Dev.* 1, 268-76).

For use in the diagnostic research discussed above, kits are also provided by the present invention. Such kits are useful for the detection of a target cellular component, which is Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, wherein the presence or an increased concentration of Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts is indicative for a prostate tumor, endometrial cancer, melanoma, chorion carcinoma or cancer of the lung, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts. The probe can be detectably labeled. Such probe may be a specific antibody or specific oligonucleotide. In a preferred embodiment, said kit contains an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a-and/or anti-Trp10b-antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said kits are based on a RIA and contain said antibody marked with a radioactive isotope. In a preferred embodiment of the kit of the invention the antibody is labeled with enzymes, fluorescent compounds, luminescent compounds, ferromagnetic probes or radioactive compounds. The kit of the invention may comprise one or more containers filled with, for example, one or more probes of the invention. Associated with container(s) of the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, us or sale for human administration.

## EXAMPLES

The following Examples are intended to illustrate, but not to limit the invention. While such Examples are typical of those that might be used, other methods known to those skilled in the art may alternatively be utilized.

### **Example 1: Materials and Methods**

#### **(A) Isolation of cDNA clones and Northern blot analysis**

Total RNA was isolated from human placenta and prostate using standard techniques. Isolation of mRNA was performed with poly (A)<sup>+</sup>RNA - spin columns (New England Biolabs, Beverly, USA) according to the instructions of the manufacturer. Poly (A)<sup>+</sup>RNA was reverse transcribed using the cDNA choice system (Gibco-BRL, Rockville, USA) and subcloned in λ-Zap phages (Stratagene, La Jolla, USA). An human expressed sequence tag (GenBank accession number 1404042) was used to screen an oligo d(T) primed human placenta cDNA library. Several cDNA clones were identified and isolated. Additional cDNA clones were isolated from two specifically primed cDNA libraries using primers 5'-gca tag gaa ggg aca ggt gg-3' and 5'-gag agt cga ggt cag tgg tcc-3'.

cDNA clones were sequenced using a thermocycler (PE Applied Biosystems, USA) and Thermo Sequenase (Amersham Pharmacia Biotech Europe, Freiburg, Germany). DNA sequences were analyzed with an automated sequencer (Licor, Lincoln, USA).

For Northern blot analysis 5 µg human poly (A)<sup>+</sup> RNA from human placenta or prostate were separated by electrophoresis on 0.8 % agarose gels. Poly (A)<sup>+</sup> RNA was transferred to Hybond N nylon membranes (Amersham Pharmacia Biotech Europe, Freiburg, Germany). The membranes were hybridized in the presence of 50 % formamide at 42°C over night. DNA probes were labelled using [ $\alpha^{32}\text{P}$ ]dCTP and the „ready prime„ labelling kit (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Commercial Northern blots were hybridized according to the distributors instructions (Clontech, Paolo Alto, USA).

#### **(B) Construction of expression plasmids and transfection of HEK 293 cells**

Lipofections were carried out with the recombinant dicistronic eucaryotic expression plasmid pdiTRP8 containing the cDNA of Trp8b under the control of the chicken β-actin promotor followed by an internal ribosome entry site (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and

the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken  $\beta$ -actin promotor. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol.Cell.Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

For monitoring of the intracellular  $\text{Ca}^{2+}$  concentration human embryonic kidney (HEK 293) cells were cotransfected with the pcDNA3-TRP8b vector and the pcDNA3-GFPvector in a molar ratio of 4 : 1 in the presence of lipofectamine (Quiagen, Hilden, Germany). To obtain pcDNA3-TRP8b the entire protein coding region of TRP8b including the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research 15, 8125-8148) was subcloned into the pcDNA3 vector (Invitrogen, Groningen, Netherlands). Calcium monitoring and patch clamp experiments were carried out two days and one day after transfection, respectively.

#### **(C) Chromosomal localization of the Trp8 gene**

The chromosomal localization of the human TRP8 gene was performed using NIGMS somatic hybrid mapping panel No.2 (Coriell Institute, Camden, NJ, USA) previously described (Drwinga, H.L., Toji, L.H., Kim, C.H., Greene, A.E., Mulivor, R.A. (1993) Genomics 16, 311-314; Dubois, B.L. and Naylor, S.L. (1993) Genomics 16, 315-319).

#### **(D) In Vitro Translation, glutathione - sepharose and calmodulin agarose binding assay**

N- and C-terminal Trp8-fragments were subcloned into the pGEX-4T2 vector (Amersham Pharmacia Europe, Freiburg, Germany) resulting in glutathione-S-transferase (GST)-Trp8 fusion constructs (Fig. 4). The GST-TRP8-fusion proteins were expressed in *E. coli* BL 21 cells and purified using glutathione - sepharose beads (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

In vitro translation of human Trp8 cDNA and *Xenopus laevis* calmodulin cDNA (Davis, T.N. and Thorner, J. Proc.Natl.Acad.Sci. USA 86, 7909-7913.) was performed in the presence of  $^{35}\text{S}$ -methionine using the TNT coupled transcription/translation kit (Promega, Madison, USA). Translation products were purified by gel filtration (Sephadex G50, Amersham Pharmacia Biotech Europe, Freiburg, Germany) and equal amounts of  $^{35}\text{S}$  labeled probes were incubated for 2 h with glutathione beads bound to GST - Trp8 or calmodulin - agarose (Calbiochem) in 50 mM Tris-HCl, pH 7.4, 0.1 % Triton X-100, 150 mM NaCl in the presence of 1 mM  $\text{Ca}^{2+}$  or 2 mM EGTA. After three washes, bound proteins were eluted with SDS sample buffer, fractionated by SDS-PAGE and  $^{35}\text{S}$  labeled proteins were detected using a Phosphor Imager (Fujifilm, Tokyo, Japan).

#### **(E) Calcium measurements**

The intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) was determined by dual wavelength fura-2 fluorescence ratio measurements (Tsien, R.Y. (1988) Trends Neurosci. 11, 419-424) using a digital imaging system (T.I.L.L. Photonics, Planegg, Germany). HEK cells were grown in minimal essential medium in the presence of 10 % fetal calf serum and cotransfected with the pcDNA3-TRP8b vector and the pCDNA3-GFPvector as described above (B). Transfected cells were detected by development of green fluorescence. The cells were loaded with 4  $\mu\text{M}$  fura-2/AM (Molecular Probes, Oregon, USA) for one hour. After loading the cells were rinsed 3 times with buffer B1 (10 mM Hepes, 115 mM NaCl, 2 mM  $\text{MgCl}_2$ , 5 mM KCl, pH 7.4) and the  $[\text{Ca}^{2+}]_i$  was calculated from the fluorescence ratios obtained at 340 and 380 nm excitation wavelengths as described (Garcia, D.E., Cavalié, A. and Lux, H.D. (1994) J. Neurosci 14, 545-553).

#### **(F) Electrophysiological recordings**

HEK cells were transfected with the eucaryotic expression plasmid pdiTRP8 described in (B) and electrophysiologigal recordings were carried out one day after transfection. Single cells were voltage clamped in the whole cell mode of the patch clamp technique as described (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100; Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. 15, 6166-6171). The pipette solution contained (mM): 140 aspartic acid, 10 EGTA, 10 NaCl, 1  $\text{MgCl}_2$ , 10 Hepes (pH 7.2 with CsOH) or 125 CsCl, 10 EGTA, 4  $\text{CaCl}_2$ , 10 Hepes (pH 7.2 with CsOH). The bath solution contained (mM): 100 NaCl, 10 CsCl, 2  $\text{MgCl}_2$ , 50 mannitol, 10 glucose, 20

Hepes (pH 7.4 with CsOH) and 2 CaCl<sub>2</sub>, or no added CaCl<sub>2</sub> (-Ca<sup>2+</sup> solution). Divalent free bath solution contained (mM): 110 N-methyl-D-glucamine (NMDG). Whole cell currents were recorded during 100 msec voltage ramps from -100 to +100 mV at varying holding potentials.

#### **(G) In Situ Hybridization**

In situ hybridizations were carried out using formalin fixed tissue slices of 6 - 8 µM thickness. The slices were hydrated and incubated in the presence of PBS buffer including 10 µg / ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 0.5 h. The slices were hybridized at 37°C using biotinylated deoxy-oligonucleotides (0.5 pmol / µl) in the presence of 33 % formamide for 12 h. Furthermore the slices were several times rinsed with 2 x SSC and incubated at 25°C for 0.5 h with avidin / biotinylated horse raddish peroxidase complex (ABC, DAKO, Santa Barbara, USA). After several washes with PBS buffer the slices were incubated in the presence of biotinylated tyramid and peroxide (0.15 % w/v) for 10 min, rinsed with PBS buffer and additionally incubated with ABC complex for 0.5 h. The slices were washed with PBS buffer and incubated in the presence of DAB solution (diaminobenzidine (50µg / ml), 50 mM Tris/EDTA buffer pH 8.4, 0.15 % H<sub>2</sub>O<sub>2</sub> in N,N - dimethyl-formamide; Merck, Darmstadt, Germany). The detection was stopped after 4 minutes by incubating the slides in water. Tyramid was biotinylated by incubating NHS-LC Biotin (sulfosuccinimidyl-6-(biotinimid)-hexanoat), 2.5 mg / ml; Pierce, Rockford, USA) and tyramin-HCl (0.75 mg / ml, Sigma) in 25 mM borate buffer pH 8.5 for 12 h. The tyramid solution was diluted 1 - 5 : 1000 in PBS buffer.

#### **(H) GenBank accession numbers: TRP8a, Aj243500; TRP8b Aj243501**

#### **Example 2: Expression of TRP8 transcripts**

In search of proteins distantly related to the TRP family of ion channels, an human expressed sequence tag (EST, GenBank accession number 1404042) was identified in the GenBank database using BLAST programmes (at the National Center for Biotechnology Information (NCBI); Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J.J. (1990) Mol. Biol. 5, 403-410) being slightly homologous to the VR1 gene. Several human placenta cDNA libraries were constructed and screeened with this EST DNA as probe. Several full length

cDNA clones were identified and isolated. The full length cDNA clones encoded two putative proteins differing in three amino acids and were termed Trp8a and Trp8b (Fig. 1c, 2a, 7 and 8A). This finding was reproduced by isolating cDNA clones from two cDNA libraries constructed from two individual placentas. The derived protein sequence(s) comprises six transmembrane domains, a characteristic overall feature of trp channels and related proteins (Fig.: 1b). The sequence is closely related to the meanwhile published calcium uptake transport protein 1 (CaT1), isolated from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A. (1999) *J Biol Chem.* 6;274, 22739-22746) and to the epithelial calcium uptake channel (ECaC) isolated from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) *J Biol Chem.* 26;274, 8375-8378). Expression of Trp8a/b transcripts are detectable in human placenta, pancreas and prostate (Fig.: 5) and the size of the Northern signal (3.0 kb) corresponds with the size of the isolated full length cDNAs. In addition, a shorter transcript of 1.8 kb, probably a splice variant, is detectable in human testis. The Trp8 mRNA is not expressed in small intestine or colon (Fig.: 5) implicating that Trp8 is not the human ortholog of the rat CaT1 or rabbit ECaC proteins. To investigate whether there are other related sequences Trp8a/b derived primers (UW241, 5'-TAT GAG GGT TCA GAC TGC-3' and UW242, 5'-CAA AGT AGA TGA GGT TGC-3') were used to amplify a 105 bp fragment from human genomic DNA being 95% identical on the nucleotide level to the Trp8 sequence (data not shown). This indicates the existence of several similar sequences in humans at least at the genomic level.

**Example 3: Two variants of the Trp8 protein (Trp8a and Trp8b) arise by polymorphism**

Two variants of the Trp8 cDNA were isolated from human placenta (Fig.: 2A, 7 and 8A) which encoded two proteins which differ in three amino acids and were termed Trp8a and Trp8b. Trp8a/b specific primers were designed to amplify a DNA fragment of 458 bp of the Trp8 gene from genomic DNA isolated from human T-lymphocytes (primer pair: UW243, 5'-CAC CAT GTG CTG CAT CTA CC-3' and UW244, 5'-CAA TGA CAG TCA CCA GCT CC-3'). The amplification product contains a part of the sequence where the derived protein sequence of Trp8a comprises the amino acid valine and the Trp8b sequence methionine as well as a silent base pair exchange (g versus a) and an intron of 303bp (Fig.: 2.A, B). Both variants of the Trp8 genes (a,b) were amplified from genomic DNA in equal amounts indicating the existence of both variants in the human genome and therefore being not the

result of RNA editing (Fig.: 2B). The Trp8a gene can be distinguished from the Trp8b gene by cutting the genomic fragment of 458bp with the restriction enzyme Bsp1286I (Fig. 2B). Using human genomic DNA isolated from blood of twelve human subjects as template, the 458bp fragment was amplified and restricted with Bsp1286I. In eleven of the tested subjects only the Trp8b gene is detectable, while one subject (7) contains Trp8a and Trp8b genes (Fig.: 2D). These implicates that the two Trp8 variants arise by polymorphism and do not represent individual genes. Using Trp8 specific primers and chromosomal DNA as template, the Trp8 locus is detectable on chromosome 7 (Fig.: 2C).

#### **Example 4: Trp8b is a calcium permeable channel**

The protein coding sequence of the Trp8b cDNA was subcloned into pcDNA3 vector (Invitrogen, Groningen, Netherlands) under the control of the cytomegalovirus promotor (CMV). Human embryonic kidney (HEK 293) cells were cotransfected with the Trp8b pcDNA3 construct (pcDNA3-Trp8b vector) and the pcDNA3-GFPvector encoding the green fluorescent protein (GFP) in 4:1 ratio. The Trp8b cDNA and the cDNA of the reporter, GFP, was transiently expressed in human embryonic kidney (HEK 293) cells. The intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) and changes of  $[\text{Ca}^{2+}]_i$  were determined by dual wavelength fura-2 fluorescence ratio measurements (Fig.: 3F) in cotransfected cells which were identified by the green fluorescence of the reporter gene GFP.

Dual wavelength fura-2 fluorescence ratio measurement is a standard procedure (e.g. in: An introduction of Molecular Neurobiology (ed. Hall, Z.W.)Sinauer Associates, Sunderland, USA (1992)) using fura-2, which is a fluorescent  $\text{Ca}^{2+}$  sensitive dye and which was designed by R.Y.Tsien (e.g. Trends Neurosci. 11, 419-424 (1988) based upon the structure of EGTA. Its fluorescence emission spectrum is altered by binding to  $\text{Ca}^{2+}$  in the physiological concentration range. In the absence of  $\text{Ca}^{2+}$ , fura-2 fluoresces most strongly at an excitation wavelength of 385 nm; when it binds  $\text{Ca}^{2+}$ , the most effective excitation wavelength shifts to 345 nm. This property is used to measure local  $\text{Ca}^{2+}$  concentrations within cells. Cells can be loaded with fura-2 esters (e.g. fura-2AM) that diffuse across cell membranes and are hydrolyzed to active fura-2 by cytosolic esterases.

In the presence of 1mM  $\text{Ca}^{2+}$ , Trp8 expressing cells typically contained more than 300 nM cytosolic  $\text{Ca}^{2+}$ , while non transfected controls contained less than 100 nM  $\text{Ca}^{2+}$  ions (Fig. 3F).

When Trp8b transfected cells were incubated without extracellular  $\text{Ca}^{2+}$ , the intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) decreased to levels comparable to non transfected cells. Readdition of 1mM  $\text{Ca}^{2+}$  to the bath resulted in significant increase of the cytosolic  $[\text{Ca}^{2+}]$  in Trp8b transfected cells, but not in controls (Fig.: 3F). After readdition of  $\text{Ca}^{2+}$  ions to the bath solution, the cytosolic  $\text{Ca}^{2+}$  concentration remains on a high steady state level in the Trp8b transfected cells.

#### **Example 5: Trp8 expressing cells show calcium selective inward currents**

To characterize in detail the electrophysiological properties of TRP8, TRP8 and GFP were coexpressed in HEK293 cells using the dicistronic expression vector pdiTRP8 and measured currents using the patch clamp technique in the whole cell mode (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) *Pflugers Arch.*, 391, 85-100).

The eucaryotic expression plasmid pdiTRP8 contains the cDNA of Trp8b under the control of the chicken  $\beta$ -actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and the GFP (Prasher, D.C. et al. (1992), *Gene* 111, 229-233), the 5' and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) *Nucleic Acids Research* 15, 8125-8148) was introduced immediately 5' of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), *Gene* 8, 193-199) downstream of the chicken  $\beta$ -actin promotor. The IRES derived from encephalomyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) *Mol. Cell. Biol.* 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) *Nature* 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

In the presence of 2 mM external calcium, Trp8b transfected HEK cells show inwardly rectifying currents, the size of which depends on the level of intracellular calcium and the electrochemical driving force. The resting membrane potential was held either at -40 mV, or, to lower the driving force for calcium influx in between pulses, at + 70 mV. Current traces

were recorded in response to voltage ramps from  $-100$  to  $+100$  mV, that were applied every second. To monitor inward and outward currents over time, we analyzed the current size at  $-80$  and  $+80$  mV of the ramps. Figure 3A shows a representative trace of the current at  $-80$  mV over time. Both at a holding potential of  $-40$  mV or at  $+70$  mV, the currents are significantly larger than in cells transfected with only the GFP containing vector (Fig.: 3E). Interestingly, after changing to a positive holding potential, current size in Trp8 transfected cells slowly increases and reaches steady state after approximately 70 seconds (Fig.: 3A). To determine the selectivity of the induced currents, we then perfused the cells with solutions that either contain no sodium, no added  $\text{Ca}^{2+}$  (Fig. 3A, C) or a sodium containing, but divalent ion free bath solution. To control for the effect of the solution change alone, we also perfused with normal bath (see puff in Fig. 3A). While removal of external  $\text{Ca}^{2+}$  completely abolishes the trp 8 induced currents - the remaining current being identical in size and shape to the control (Fig.: 3A, C, E), removal of external sodium has no effect (Fig.: 3E). An important hallmark of calcium selective channels (e.g. Vennekens, R., Hoenderop, G.J., Prenen, J., Stuiver, M., Willems, PHGM, Droogmans, G., Nilius, B. and Bindels, R.J.M (1999) *J. Biol. Chem.* 275, 3963-3969), is their ability to conduct sodium only if all external divalent ions, namely  $\text{Ca}^{2+}$  and magnesium are removed. To test whether the trp 8 channel conforms with this phenomenon normal bath solution was switched to a solution containing only sodium and 1 mM EGTA. As can be seen in Figure 3B and D, Trp8 transfected cells can now conduct very large sodium currents. Interestingly, immediately after the solution change, the currents first become smaller before increasing rapidly, indicating that the pore may initially still be blocked by calcium a phenomenon usually called anomalous mole fraction behaviour (Warnat, J., Philipp, S., Zimmer, S., Flockerzi, V., and Cavalié A. (1999) *J. Physiol. (Lond)* 518, 631-638). The measured outward currents of Trp8 transfected cells in normal bath solution are not significantly different from non-transfected control cells or cells which only express the reporter gene GFP. As the removal of external  $\text{Ca}^{2+}$  abolishes the Trp8 specific current, the remaining current was subtracted from the current before the solution change to obtain the uncontaminated Trp8 conductance (see inset in Fig.: 3C). As expected from the given ionic conditions (high EGTA inside, 2 mM  $\text{Ca}^{2+}$  outside), the current-voltage relationship now shows prominent inward rectification with little to no outward current.

Both the time course of the development of Trp8 currents and the size of the currents depend on the frequency of stimulation (data not shown), the internal and external  $\text{Ca}^{2+}$  concentration

and the resting membrane potential, suggesting that Trp8 calcium conductance is intricately regulated by a  $\text{Ca}^{2+}$  mediated feedback mechanisms.

#### **Example 6: $\text{Ca}^{2+}$ / calmodulin binds to the C-terminus of the Trp8 protein**

To test whether calmodulin, a prime mediator of calcium regulated feedback, is involved, first it was investigated biochemically whether Trp8 protein can bind calmodulin. Trp8 cDNA was in vitro translated in the presence of  $^{35}\text{S}$ -methionine and the product incubated with calmodulin-agarose beads. After several washes either in the presence or absence of  $\text{Ca}^{2+}$ , the beads were incubated in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis. In the presence of  $\text{Ca}^{2+}$  (1mM), but not in the absence of  $\text{Ca}^{2+}$ , Trp8 protein binds to calmodulin (Fig.: 4B).

To narrow down the binding site, two approaches were undertaken: Firstly, GST-TRP8 fusion proteins of various intracellular domains of Trp8 were constructed, expressed in *E. coli* and bound to glutathione sepharose beads. These beads were then incubated with in vitro translated  $^{35}\text{S}$ - labeled calmodulin, washed and subjected to gel electrophoresis. Secondly, truncated versions of in vitro translated Trp8 protein were used in the above described binding to calmodulin-agarose. As shown in Figure 4A, and C, fusion proteins of the N-terminal region (N1, N2) of Trp8 did not bind calmodulin, while C-terminal fragments (C1, C2, C3, C4) showed calmodulin binding in the presence of calcium (for localization of fragments within the entire Trp8 protein see Fig. 4C). Accordingly, a truncated version of in vitro translated Trp8, which lacks the C-terminal 32 amino acid residues did not bind to calmodulin-agarose (4B). We have restricted the calmodulin binding site to amino acid residues 691 to 711 of the Trp8 protein. This calmodulin binding site does not resemble the typical conserved IQ - motif of conventional myosins, but has limited sequence homology to the calcium dependent calmodulin binding site 1 of the transient receptor potential like (trpl) protein of *Drosophila melanogaster* (Warr and Kelly, 1996) with several charged amino acid residues conserved. The sequence of the calmodulin binding site of the Trp8 protein resembles a putative amphipathic  $\alpha$ -helical wheel structure with a charged and a hydrophobic site according to a model proposed by Erickson-Vitanen and De Grado (1987, Methods Enzymol. 139, 455-478.).

**Example 7: Expression of Trp8 transcripts in human placenta and pancreas**

Several slides from a human placenta of a ten week old abort were used for in situ hybridization experiments. The in situ hybridization experiments revealed expression of Trp8 transcripts in human placenta (Fig.: 5B). Expression was detectable in trophoblasts and syncytiotrophoblasts of the placenta, but not in Langhans cells.

Trp8 transcripts are detectable in human pancreas (Fig.: 5A). Therefore Trp8 probes were hybridized to tissue sections of human pancreas. The pancreatic tissues were removed from patients with pancreas cancer. Trp8 expression is detectable in pancreatic acinar cells, but not in Langerhans islets (Fig.: 5C). No Trp8 expression was found in regions of pancreatic carcinomas (data not shown).

Furthermore, the Trp8 cDNA is not detectable in human colon nor in human kidney by in situ hybridization as well as by Northern analysis (Fig.: 5A, D). The Northern results taken together with the in situ expression data indicate that the Trp8 protein is not the human ortholog of the CaT1 and ECaC channels cloned from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A. (1999) J Biol Chem. 6;274, 22739-22746) and from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378), respectively. Trp8 is unlikely to represent the human version of CaT1 as its expression is undetectable in the small intestine and colon tissues where CaT1 is abundantly expressed. If, however, Trp8 is the human version of rat CaT1, a second gene product appears to be required for  $\text{Ca}^{2+}$  uptake in human small intestine and colon attributed to CaT1 in rat small intestine and colon.

**Example 8: Differential expression of Trp8 transcripts in benign and malign tissue of the prostate**

The Trp8 transcripts are expressed in human prostate as shown by hybridization of a Trp8 probe to a commercial Northern blot (Clontech, Palo Alto, USA) (Fig.: 5A). Trp8 transcripts were not detectable by Northern blot analysis using pooled mRNA of patients with benign prostatic hyperplasia (BPH) (Fig.: 5A, prostate\*). To examine Trp8 expression on the cellular

level, sections of prostate tissues were hybridized using Trp8 specific cDNA probes (Table 3). Expression of Trp8 transcripts is not detectable in normal prostate (n = 3), benign hyperplasia (BPH, n = 15) or prostatic intraepithelial neoplasia (PIN, n = 9) (Fig.: 6A, C, E). Trp8 transcripts were only detectable in prostate carcinoma (PCA), although with different expression levels. Low expression levels were found in primary carcinomas (2 - 10 % of the carcinoma cells, n = 8) (Fig.: 7B) . Much stronger expression was detectable in rezidive carcinoma (10 - 60 %) (Fig.: 7D, n = 6) and metastases of the prostate (60 - 90 %, n = 4) (Fig.: 7F). Thus it has to be concluded that the commercial Northern blot used in Fig.: 5A contains not only normal prostate mRNA as indicated by the distributor. According to the distributors instructions the prostate mRNA used for this Northern blot was collected from 15 human subjects in the range of 14 to 60 years of age. This prostate tissue was not examined by pathologic means. Since Trp8 expression is not detectable in normal or benign prostate, this finding implicates that the mRNA used for this Northern blot was extracted in part from prostatic carcinoma tissue. To summarize, Trp8 expression is only detectable in malign prostate and, thus, the Trp8 cDNA is a marker for prostate carcinoma. The results are summarized in Table 4.

Table 3

Trp8 probes used for in situ hybridization:

Probes (antisense)

- 1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'
- 2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'
- 3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

- 1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'
- 2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'
- 3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Table 4

Prostate	total	negative	positive
normal	3	3	0
BPH	15	15	0
PIN	9	9	0

carcinoma

18

1

17

**(B) Differential expression of Trp8 transcripts in benign and malign tissue of the uterus**

Moreover it could be shown that Trp8 is expressed in endometrial cancer (also called cancer of the uterus, to be distinguished from uterine sarcoma or cancer of the cervix) whereas no expression was observed in normal uterus tissue. Thus, Trp8 also is a specific marker for the diagnosis of the above cancer (Fig. 12).

**Example 9: Characterization of Trp9**

The complete protein coding sequence of Trp9 was determined (Fig. 9). Trp 9 transcripts are predominantly expressed in the human prostate and in human colon. As it could be shown by Northern blot analysis, there is no difference of the expression of TRP9 in benign prostate hyperplasia (BPH, Fig. 13, upper panel left) or prostate carcinoma (Fig. 13, upper panel right). However, Trp9 is useful as a reference marker for prostate carcinoma, i.e. can be used for quantifying the expression level of Trp8. The ratio of the expression of Trp8:Trp9 in patients and healthy individuals is useful for the development of a quantitative assay.

**Example 10: Characterization of Trp10**

The complete protein coding sequence of TRP10 (a and b) was determined by biocomputing (Fig. 10 and 11). Using a 235 bp fragment of the Trp10 cDNA as probe in Northern blot analysis TRP10 transcripts could only be detected in mRNA isolated from individuals with prostate cancer (Fig. 13, bottom panel) but not in mRNA isolated from benign tissue of the prostate (prostate BPH) nor in mRNA isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 235 bp cDNA fragment of the Trp10 cDNA was amplified using the primer pair UW248 5'-ACA GCT GCT GGT CTA TTC C-3' and UW249 5'-TAT

GTG CCT TGG TTT GTA CC-3' and prostate cDNA as template. In summary, Trp10a and Trp10b, like TRP8 are also expressed in malignant prostate tissue. So far, its expression could not be observed in any other tissue examined (see above). Thus, Trp 10a and Trp10b are also useful markers which are specific for malignant prostate tissue.

Furthermore, database searches in public databases of the national center for biological information (NCBI) revealed the existence of several expressed sequence tags (EST clones) being in part identical to the Trp10 sequence. These EST clones were originally isolated from cancer tissues of lung, placenta, prostate and from melanoma. These clones include the clones with the following accession numbers: BE274448, BE408880, BE207083, BE791173, AI671853, BE390627. The results demonstrate that cancer cells of these tissues express Trp10 related transcripts whereas no expression of Trp10 transcripts in the corresponding healthy tissues are detectable (Figure 13). Furthermore, it could be shown that in cancer cells of melanoma and prostate cancer Trp10 transcripts are expressed as shown by in situ hybridizations using 4 antisense probes (Figure 14A – E and 13K-O and Table 2, above). Furthermore, it could clearly be shown that cancer cells of these tissues expressing Trp10 transcripts also express Trp10-antisense transcripts as shown in Figure 14F-J, Figure 14P-R and Figure 14T by in situ hybridizations using 4 sense probes (Table 2, above). The in situ hybridization experiments demonstrate that detection of a subset of cancer cells derived from carcinoma of lung, placenta, prostate and melanoma is feasible using antisense as well as sense probes complementary to Trp10 transcripts or complementary to Trp10-antisense transcripts, respectively.

The foregoing is meant to illustrate but not to limit the scope of the invention. The person skilled in the art can readily envision and produce further embodiment, based on the above teachings, without undue experimentation.

**What Is claimed Is:**

1. An isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of
  - (a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11;
  - (b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11;
  - (c) a nucleic acid molecule included in DSMZ Deposit No. DSM 13579, DSM 13580, DSM 13584, DSM 13581 or DSM....;
  - (d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c);
  - (e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
  - (f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).
2. A recombinant vector containing the nucleic acid molecule of claim 1
3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.
4. A recombinant host cell which contains the recombinant vector of claim 3.
5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
6. An isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b which is encoded by a nucleic acid molecule of claim 1.
7. A recombinant host cell that expresses the isolated protein of claim 6.

8. A method of making an isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising:
  - (a) culturing the recombinant host cell of claim 6 under conditions such that said protein is expressed; and
  - (b) recovering said protein.
9. The protein produced by the method of claim 8.
10. An antisense RNA sequence characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to said mRNA or part thereof, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
11. A ribozyme characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to and cleave said mRNA or part thereof, thus inhibiting the synthesis of the protein encoded by said nucleic acid molecule.
12. An inhibitor characterized in that it can suppress the activity of the protein of claim 6.
13. A method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.
14. The method of claim 13, wherein the reagent is a nucleic acid.
15. The method of claim 13, wherein the reagent is an antibody.
16. The method of claim 13, wherein the reagent is detectably labeled.

17. The method of claim 16, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
18. A method for diagnosing an endometrial cancer (carcinoma of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the Trp8a and/or Trp8a and/or trp8b encoding mRNA and detecting Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA.
19. The method of claim 18, wherein the reagent is a nucleic acid.
20. The method of claim 18, wherein the reagent is an antibody.
21. The method of claim 18, wherein the reagent is detectably labeled.
22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
23. A method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA or Trp10a and/or Trp10b related antisense RNA.
24. A method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (carcinoma of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a therapeutically effective amount of a reagent which decreases or inhibits expression of Trp8a, Trp8b, Trp10a and/or Trp10b and/or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b.
25. The method of claim 24, wherein the reagent is a nucleotide sequence comprising an antisense RNA.

26. The method of claim 24, wherein the reagent is a nucleotide sequence comprising a ribozyme.
27. The method of claim 24, wherein the reagent is an inhibitor of Trp8a, Trp8b, Trp10a and/or Trp10b.
28. The method of claim 27, wherein the reagent is an anti-Trp8a-, anti Trp8b-, anti-Trp10a-and/or anti-Trp10b antibody or a fragment thereof.
29. A diagnostic kit useful for the detection of Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts in a sample, wherein the presence of an increased concentration of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts is indicative for a prostate tumor, endometrial cancer (cancer of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts.
30. The kit of claim 29, wherein the target component to be detected is Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b and the probe is an antibody.
31. A method for identifying a compound which acts as an agonist or antagonist on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

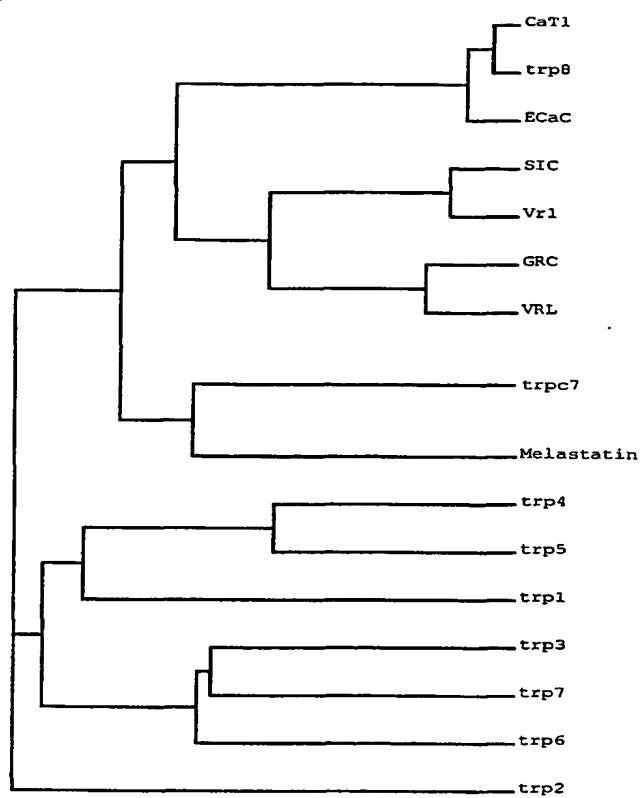
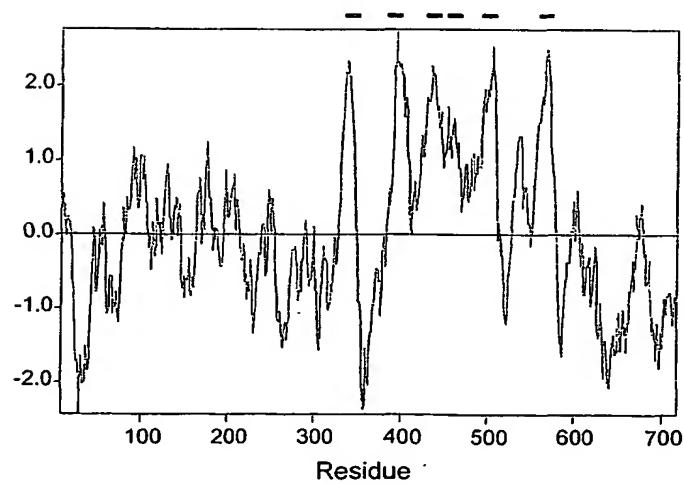
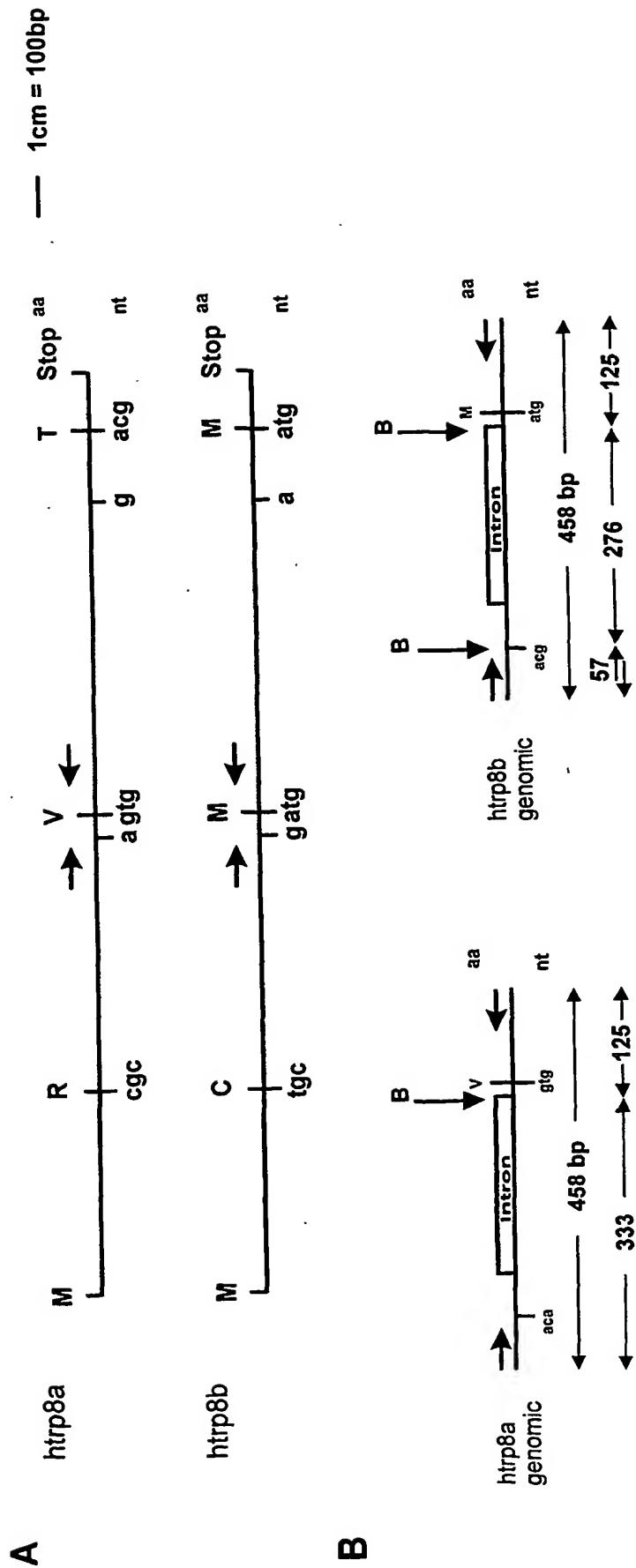
**Figs. 1A and 1B****A****B**

Fig. 1C

C

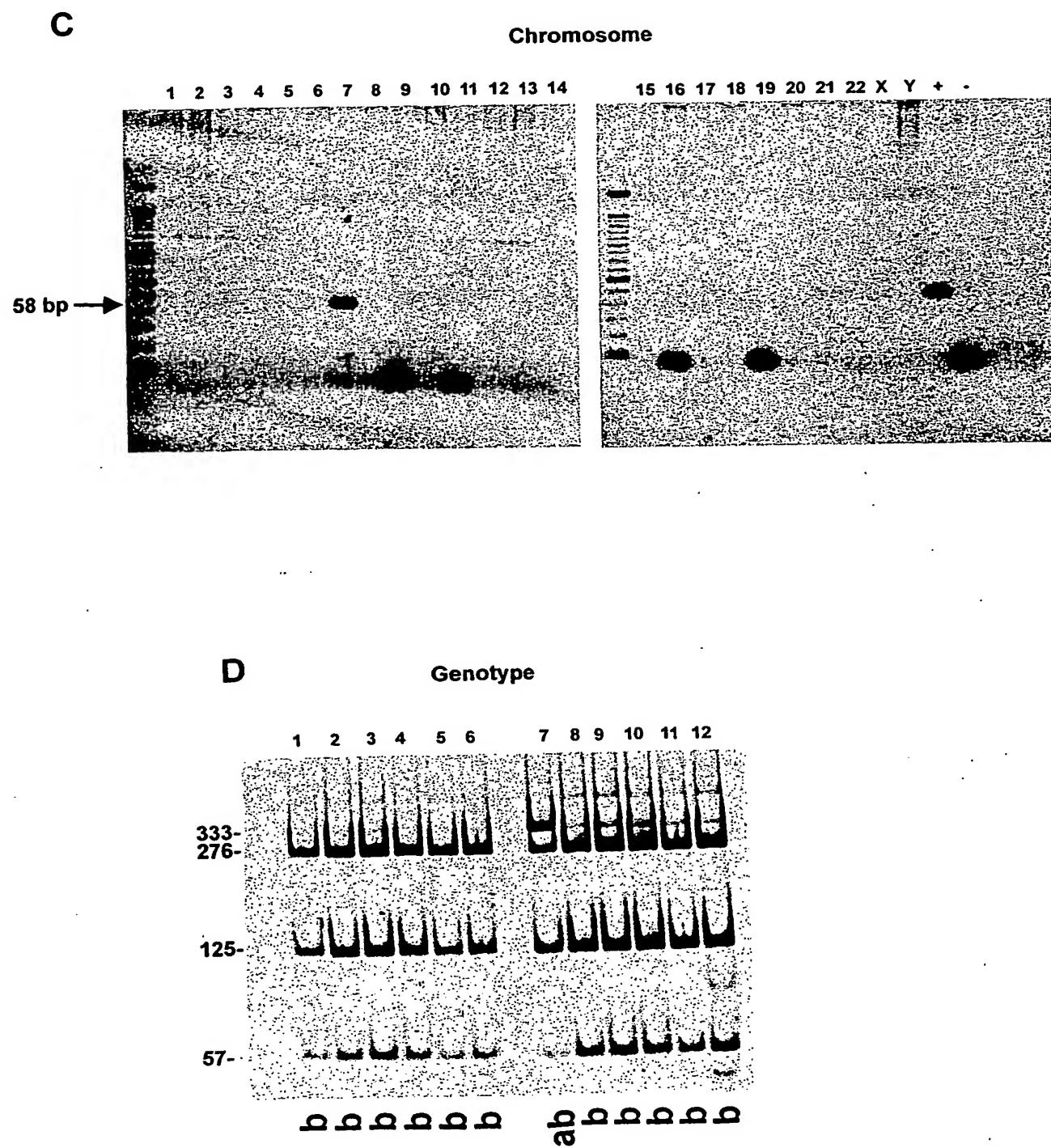
htrp8A		MG	2
htrp8B		MG	2
Vr1	MEQRASLDSEESESPPQENSCLDPDRDPNCKPPVKPHI	MG	60
ECaC	FTTRSR7RLFGKGDSEASP	MG	2
htrp8A	LISLKEKG-----LILCILSKFCRWFQRR	ESWAQSRDEONLILQOK-RIWEsp-LIL	52
htrp8B	LISLKEKG-----LILCILSKFCRWFQRR	ESWAQSRDEONLILQOK-RIWEsp-LIL	52
Vr1	LDCPVEEGLASCPIITVSSVLTIQPGDGPASVRPSSQDSVSAGEKPPRLYDRRSIFDA	120	
ECaC	ACPEKAG-----PWAOLKLLISWVGE	QDWEQYRDRVNMLQEE-HIRDsp-LIQ	52
htrp8A	AKNDVQAIKNLILYEDCKVH-----ORGAMGETAKHIAAH-YDN	LEAMVYMEAR	102
htrp8B	AKNDVQAIKNLILYEDCKVH-----ORGAMGETAKHIAAH-YDN	LEAMVYMEAR	102
Vr1	VAQSNCOEFLSPELORSKRTDSEFKDPETGTCILGMNLSLNGNDTIALLLOVA	180	
ECaC	AKENDLRLKILILUNOSCFO-----ORGAVGETAKHIAAH-YDN	LEATLIMEAR	102
htrp8A	-----PELVFEPMTSELVEGQALHIIWVWONNNVYRAILARRASPSRPAIGTAAERSP	156	
htrp8B	-----PELVFEPMTSELVEGQALHIIWVWONNNVYRAILARRASPSRPAIGTAAERSP	156	
Vr1	RKTDLSLKGQFVNASYTDSYYKGQALHIIWVWONNNVYRAILARRASPSRPAIGTAAERSP	240	
ECaC	-----PELAKEPALCEPFGQTAIILQJLWVQMCNLYWRAILARRASPSRPAIGTAAERSP	156	
htrp8A	-----INLIFCEHEHLSFIAQVNSEEETVRLLIEHG-----ADIRADSIGNTVHILQ	207	
htrp8B	-----INLIFCEHEHLSFIAQVNSEEETVRLLIEHG-----ADIRADSIGNTVHILQ	207	
Vr1	GRPGFVFGSILMSIAACTNOLATVKFLNLSWPAQDLSRSVNTVHILVEADNTVD	300	
ECaC	-----INLIFCEHEHLSFIAQVNSEEETVRLLIEHG-----ADIRADSIGNTVHILQ	207	
htrp8A	-----NKTACOMVNLILSSEYDRHGDHLQPDILVPHQGLTFIQLAGVEANTVMPHQI	261	
htrp8B	-----NKTACOMVNLILSSEYDRHGDHLQPDILVPHQGLTFIQLAGVEANTVMPHQI	261	
Vr1	NTKFTVSKYNEIKILGAKLHPTLKGEEETRKGLTLELAASSKGKGLVAYILQREIHEP	360	
ECaC	-----NKTACOMVNLILSSEYDRHGDHLQPDILVPHQGLTFIQLAGVEANTVMPHQI	261	
htrp8A	-----KRNHTOTYGRILSTLDETEOSSGDECISLLELLITTK-KREAR-OI6DOTEVK	314	
htrp8B	-----KRNHTOTYGRILSTLDETEOSSGDECISLLELLITTK-KREAR-OI6DOTEVK	314	
Vr1	ECRHLISRKFTRRAYGEVHSLSYDLSCTIC-EKNSVLEWIAVYSETPNRHMILVERIN	420	
ECaC	-----KRNHTOTYGRILSTLDETEOSSGDECISLLELLITTK-KREAR-OI6DOTEVK	314	
htrp8A	-----EVTSLIKQKRYGRPFQMLGAIIILYICETMCCIIAPLKPRTNRNTSFNDNTLLOQKLIQ	374	
htrp8B	-----EVTSLIKQKRYGRPFQMLGAIIILYICETMCCIIAPLKPRTNRNTSFNDNTLLOQKLIQ	374	
Vr1	RLILODKNDRFVKRIFYFVNFFVQCLYMIITAAAYVYREVEG---LPP-----YKLN	468	
ECaC	-----EVTSLIKQKRYGRPFQMLGAIIILYICETMCCIIAPLKPRTNRNTSFNDNTLLOQKLIQ	374	
51			
htrp8A	EAVYTPKODIRLVEGLTVIGAIILILVVEVPDIFRMGVTRFFGQTIILGGPFHVLIIITYAF	434	
htrp8B	EAVYTPKODIRLVEGLTVIGAIILILVVEVPDIFRMGVTRFFGQTIILGGPFHVLIIITYAF	434	
Vr1	-----TVCQYFVIGEILSVSSGGVYFFRFGQIYFLORRPS---LKSLEVDSYSEILFFVOSL	522	
ECaC	EAVYTPKODIRLVEGLTVIGAIILILVVEVPDIFRMGVTRFFGQTIILGGPFHVLIIITYAS	434	
52			
htrp8A	MVLTVMYMRLLISASEGUUVPMSEALVIGCNVMSFARGFOMLSPFTIMIOKMIFGDLMR	494	
htrp8B	MVLTVMYMRLLISASEGUUVPMSEALVIGCNVMSFARGFOMLSPFTIMIOKMIFGDLMR	494	
Vr1	FMLVSVLYLFSORKEWASMSVLSIAMPITMLYITRGEQOMGIYAVMIEKMLRDLCRM	582	
ECaC	LVLTLMYMRLLTNNINGEVVPLSEALVIGCNVMSFARGFOMLSPFTIMIOKMIFGDLMR	494	
54			
htrp8A	WLMAVVILGEASAFYIIFQTED---FEE-----LG-HFYDYPALFSTELV	538	
htrp8B	WLMAVVILGEASAFYIIFQTED---FEE-----LG-HFYDYPALFSTELV	538	
Vr1	FVLYVFLFGFSTAVVTLIEDGKNNLSMESTPHKCRGSACKPGENSYNSLYSTCLELKF	642	
ECaC	WLMAVVILGEASAFHITFQTED---ENN-----LG-EFSDYPTALESTGELE	538	
55			
htrp8A	LTTIIGPANYNVVLDPMWVSTYAFATLTLIMNLLIMHEDPHWRVVAHERDELRAQI	598	
htrp8B	LTTIIGPANYNVVLDPMWVSTYAFATLTLIMNLLIMHEDPHWRVVAHERDELRAQI	598	
Vr1	IGMGLDELTENTYDFKAVLILLLQAYVLLTILLLMLLALGEETVNLKRSKNNKLQR	702	
ECaC	LTTIIGPANYSVVLDPMWVSTYAFATLTLIMNLLIMHEDPHWRVVAHERDELRAQV	598	
56			
htrp8A	VATTVMLERKLPRCLWP--RSG---ICGREYGLCD--RWFLEVEDRODILNARQIORYAOA	671	
htrp8B	VATTVMLERKLPRCLWP--RSG---ICGREYGLCD--RWFLEVEDRODILNARQIORYAOA	671	
Vr1	AITILDTEKSFLKCRMKAFRSGKLLQWGTGFTPDGKDDYKRCFVDEVNWTWNTNGVIIINE	762	
ECaC	VATTVMLERKLPRCLWP--RSG---ICGREYGLCD--RWFLEVEDRODILNARQIORYEA	671	
57			
htrp8A	FHTR---ESEDLQDSV-EKLELCPFSPHLSL-----PSVSRSTSRSSANVERLROGLTLLR	726	
htrp8B	FHTR---ESEDLQDSV-EKLELCPFSPHLSL-----PSVSRSTSRSSANVERLROGLTLLR	726	
Vr1	DPGN---CEGVKRTLSFSLRSGRVSQRNWMFALVPLLRDASTRDRHATQOEVOLGHYTG	820	
ECaC	FNCSDKEDQEOISEKRP-STVSEQMSASRASVAFQTPLSLRTSQSSN---SHRGWEILR	728	
58			
htrp8A	DLRGIIINRGLLEDGESWEYQI*	746	
htrp8B	DLRGIIINRGLLEDGESWEYQI*	746	
Vr1	SIMPEDAEVFKDSMVPGEK*	839	
ECaC	NTLGHINLGLDLGEGDGEEVYHF*	751	

### Figs. 2A and 2B

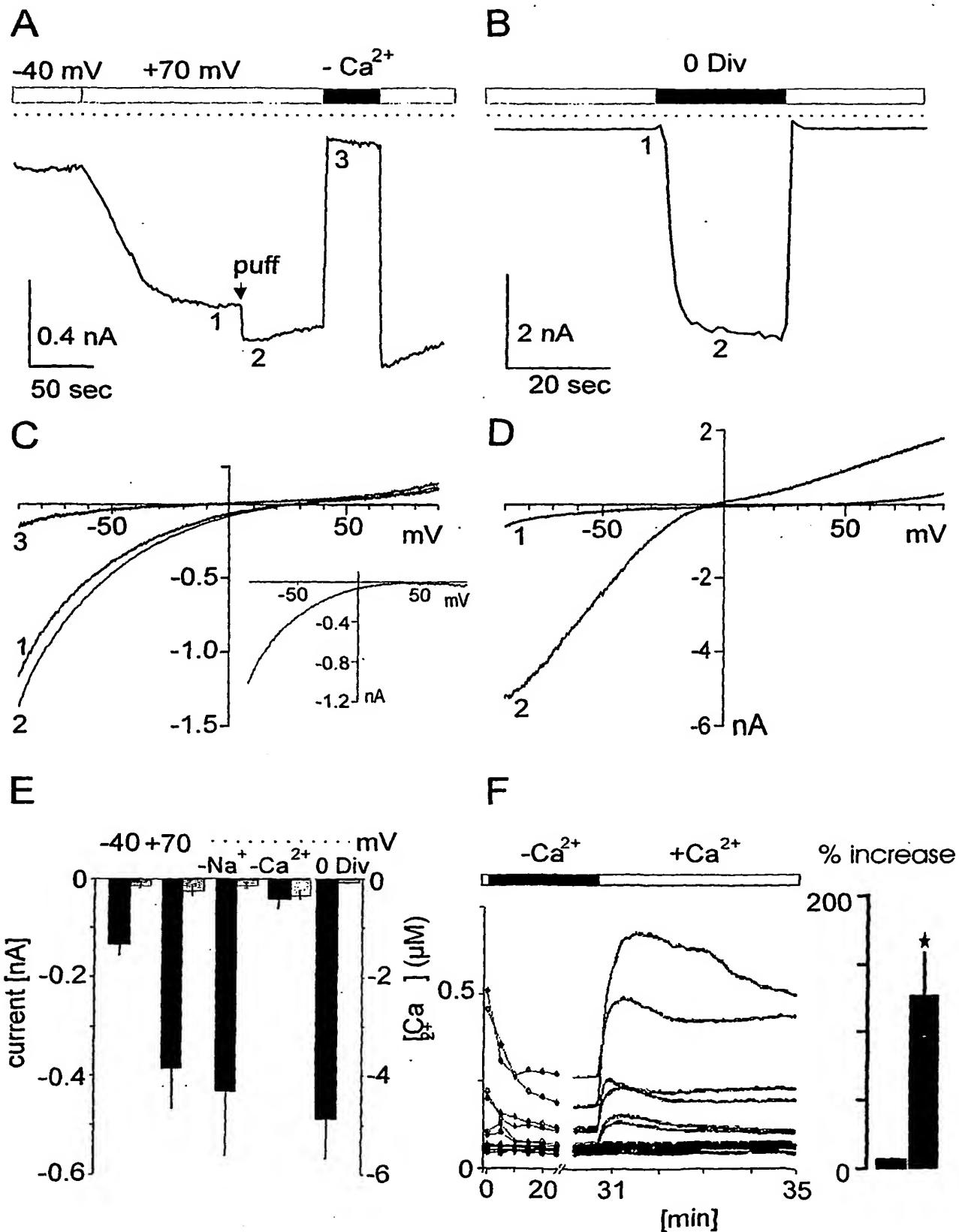


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Figs. 2C and 2D



Figs. 3A – 3F



Figs. 4A-4C

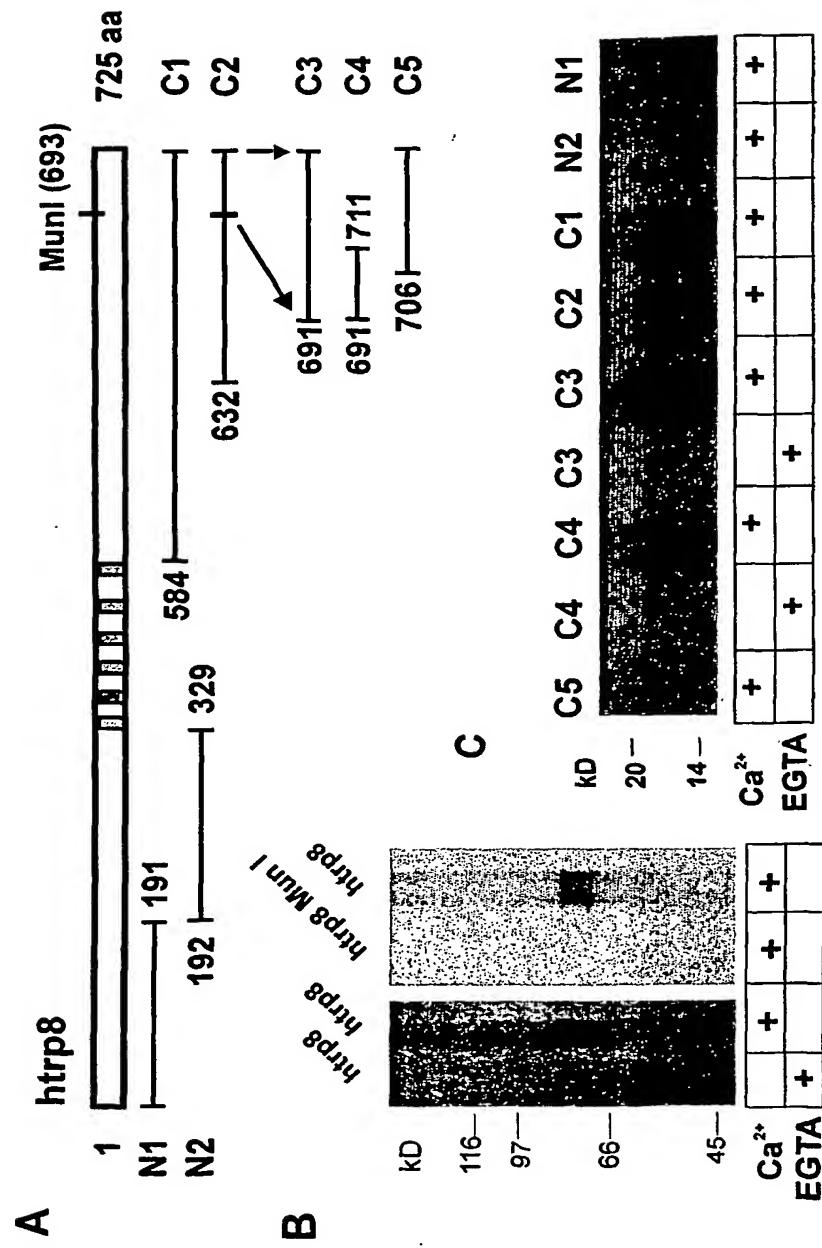
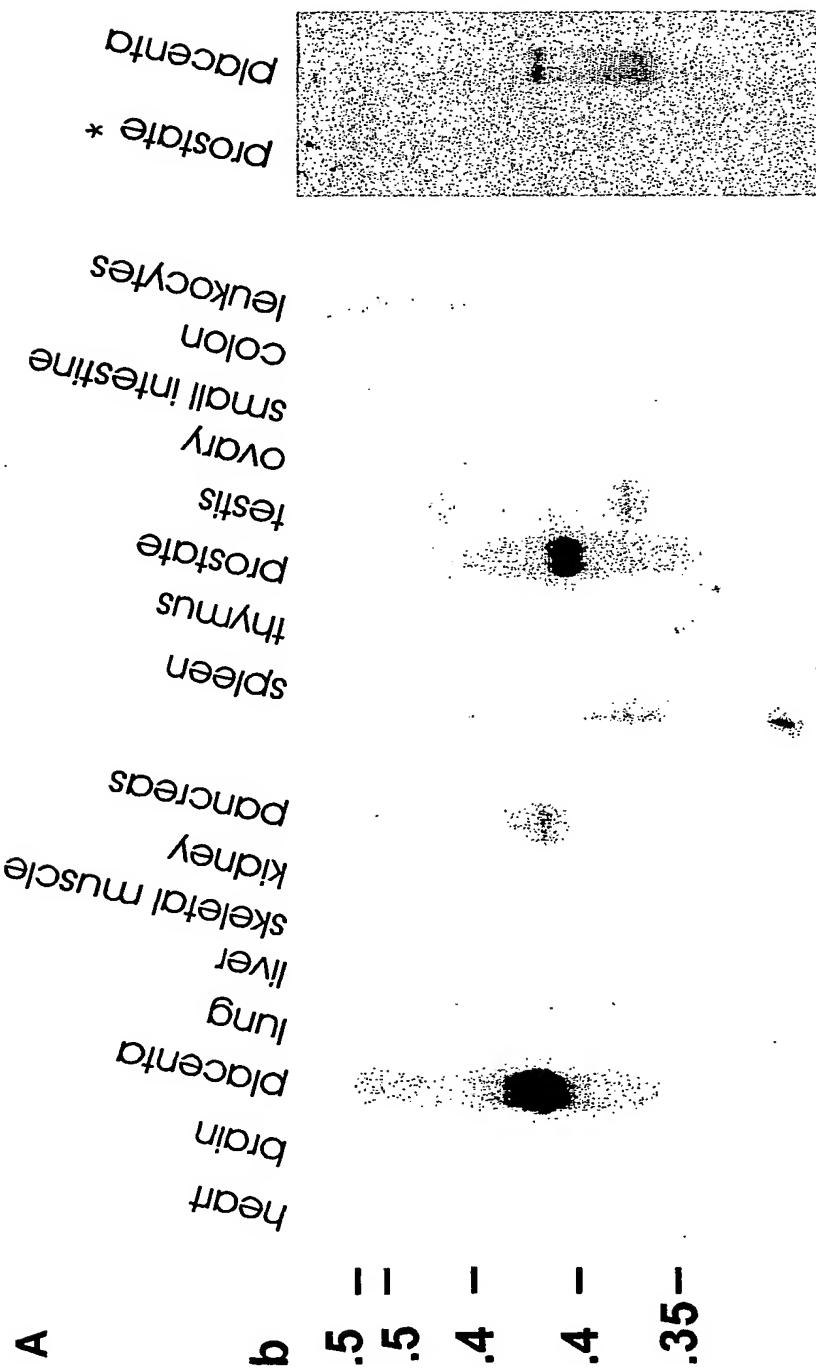
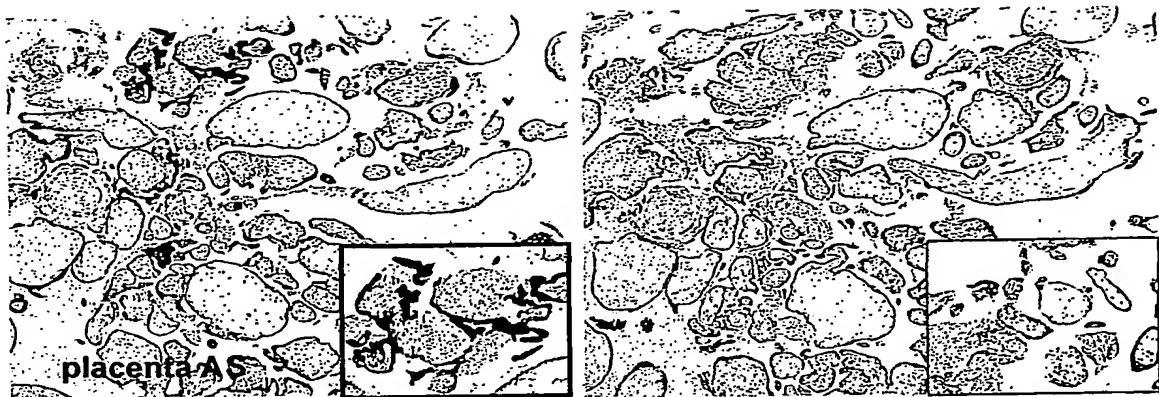
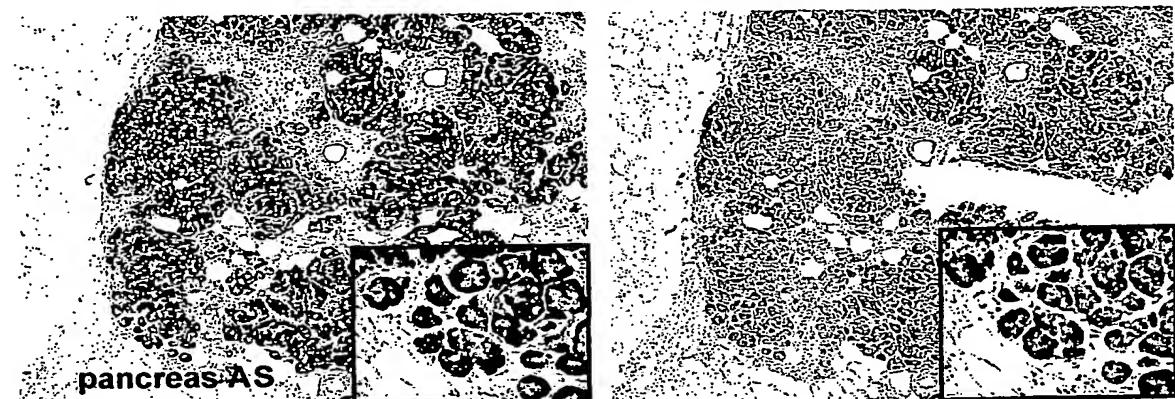
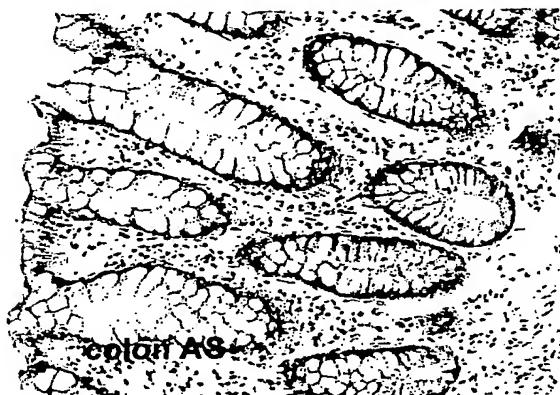


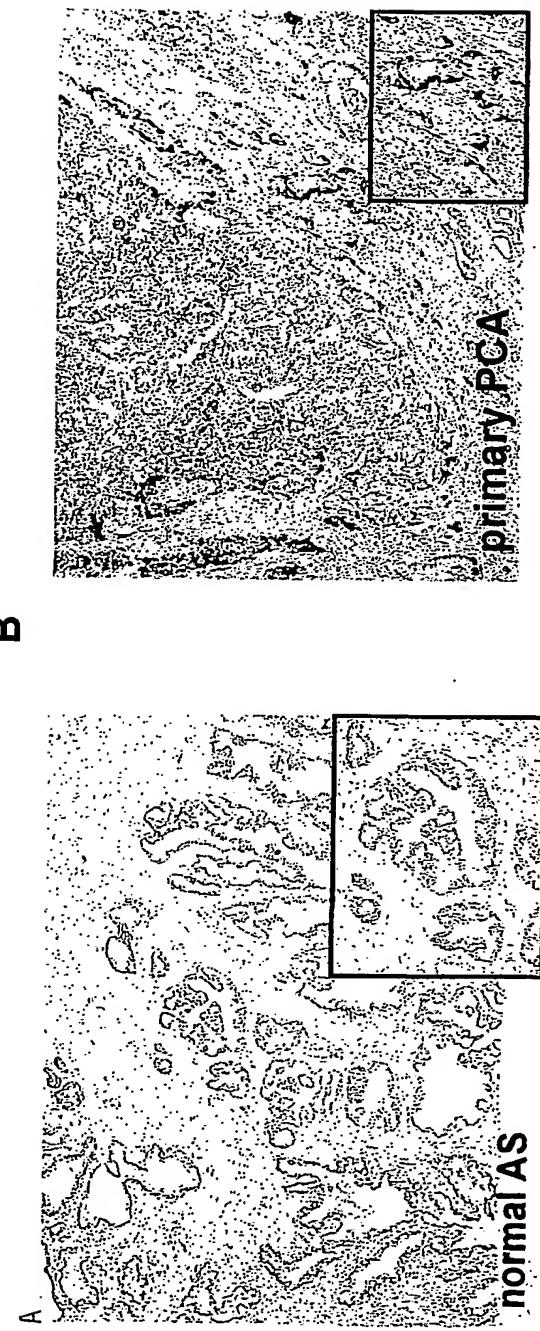
Fig. 5A



Figs. 5B – 5D

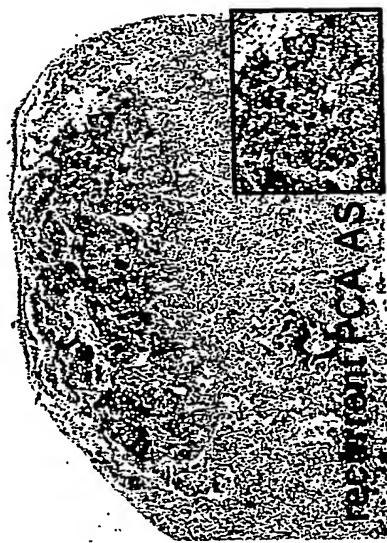
**B****C****D**

Figs. 6A and 6B

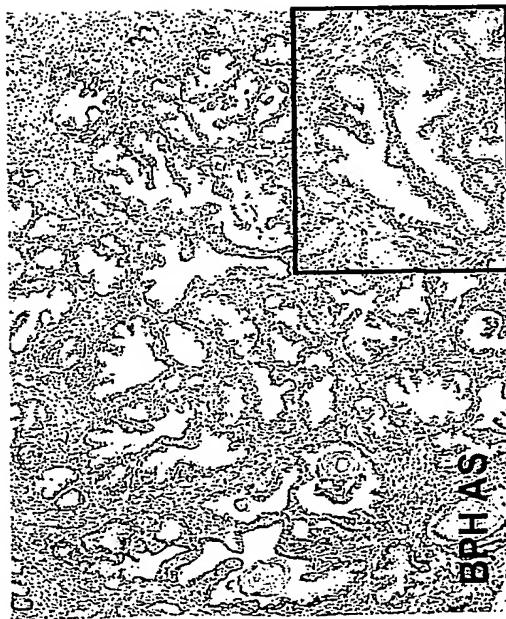


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Figs. 6C - 6F



D



E



F

10 30 50  
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 70 90 110  
 AGGAACCTCGTCAGGAAGGCAGGAGACAGGGAGACGGGACTCTACAGGGAGACGGTGGGCC  
 130 150 170  
 GGCCCTTGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCCTCAGGGTCTGGCCTCGGCC  
 190 210 230  
 TCAGGGCCCCAAGGAGCCGGCCCTACACCCATGGTTGTCACTGCCCAAGGAGAAAGG  
 250 270 290  
 M G L S L P K E K G  
 GCTAATTCTCTGCCTATGGAGCAAGTTCTGCAGATGGTCCAGAGACGGGAGTCCTGGGC  
 L I L C L W S K F C R W F Q R R E S W A  
 310 330 350  
 CCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCCTCTCCT  
 Q S R D E Q N L L Q Q K R I W E S P L L  
 370 390 410  
 TCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAAGTTGCTCAAGTATGAGGATTG  
 L A A K D N D V Q A L N K L L K Y E D C  
 430 450 470  
 CAAGGTGCACCAGAGAGGAGCCATGGGGAAACAGCGCTACACATAGCAGCCCTATGA  
 K V H Q R G A M G E T A L H I A A L Y D  
 490 510 530  
 CAACCTGGAGGCCATGGTGGTGTGGAGGCTGCCAGCTGGTCTTTGAGCCAT  
 N L E A A M V L M E A A P E L V F E P M  
 550 570 590  
 GACATCTGAGCTCTATGAGGGTCAGACTGCACATCGCTGTTGTGAACCAGAACAT  
 T S E L Y E G Q T A L H I A V V N Q N M  
 610 630 650  
 GAACCTGGTGCAGGCCCTGCTGCCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCAC  
 N L V R A L L A R R A S V S A R A T G T  
 670 690 710  
 TGCCTTCCGCCGTAGTCCCCGCAACCTCATCTACTTTGGGGAGCACCCTTGTCCTTGC  
 A F R R S P R N L I Y F G E H P L S F A  
 730 750 770  
 TGCCTGTGTGAACAGTGGAGGAGATCGTGGCTGCTCATGGAGCATGGAGCTGACATCCG  
 A C V N S E E I V R L L I E H G A D I R  
 790 810 830  
 GGGCCAGGACTCCCTGGGAAACACAGTGTACACATCCTCATCCCTCAGGCCAACAAAC  
 A Q D S L G N T V L H I L I L Q P N K T  
 850 870 890  
 CTTTGCCCTGCCAGATGTACAACCTGTTGCTGTCCTACGACAGACATGGGGACCACCTGCA  
 F A C Q M Y N L L L S Y D R H G D H L Q  
 910 930 950  
 GCCCCCTGGACCTCGTGCCAATCACCAAGGGCTCACCCCTTCAAGCTGGCTGGAGTGG  
 P L D L V P N H Q G L T P F K L A G V E  
 970 990 1010  
 GGGTAAACACTGTGATGTTCAAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTA  
 G N T V M F Q H L M Q K R K H T Q W T Y  
 1030 1050 1070  
 TGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCA  
 G P L T S T L Y D L T E I D S S G D E Q  
 1090 1110 1130  
 GTCCCTGCTGGAACTTATCATCACCAAGAAGCGGGAGGCTGCCAGATCTGGACCA  
 S L L E L I I T T K K R E A R Q I L D Q  
 1150 1170 1190  
 GACGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAAGCGGTACGGCGGCCGTACTCTG  
 T P V K E L V S L K W K R Y G R P Y F C  
 1210 1230 1250  
 CATGCTGGGTGCCATATATCTGCTGTACATCATGCTTCACCATGTGCTGCATCTACCG  
 M L G A I Y L L Y I I C F T M C C I Y R  
 1270 1290 1310

CCCCCCTCAAGCCCAGGACCAATAACCGCACAAGCCCCGGGACAAACACCCCTCTTACAGCA  
 P L K P R T N N R T S F R D N T L L Q Q  
 1330 1350 1370  
 GAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGAGCT  
 K L L Q E A Y V T P K D D I R L V G E L  
 1390 1410 1430  
 GGTGACTGTCATTGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAAT  
 V T V I G A I I I L L V E V P D I F R M  
 1450 1470 1490  
 GGGGGTCACTCGCTTCMTGGACAGACCATCCCTGGGGGCCATTCATGTCCTCATCAT  
 G V T R F F G Q T I L G G P F H V L I I  
 1510 1530 1550  
 CACCTATGCCCTCATGGTGCCTGGTACATGGTGATGCCGCTCATCAGTGCCAGGGGA  
 T Y A F M V L V T M V M R L I S A S G E  
 1570 1590 1610  
 GGTGGTACCCATGTCCTTGCACTCGTGCTGGCTGGTGCACCGTCATGTACTTCGCCCG  
 V V P M S F A L V L G W C N V M Y F A R  
 1630 1650 1670  
 AGGATTCCAGATGCTAGGCCCTTCACCATCATGATTCAAGAGATGATTTGGCGACLT  
 G F Q M L G P F T I M I Q K M I F G D L  
 1690 1710 1730  
 GATGCGATTCTGCTGGCTGATGGCTGTGGTCATCTGGCTTGCCTCAGCCCTCTATAT  
 M R F C W L M A V V I L G F A S A F Y I  
 1750 1770 1790  
 CATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCATGGCCCT  
 I F Q T E D P E E L G H F Y D Y P M A L  
 1810 1830 1850  
 GTTCAGCACCTTCGAGCTTCCCTTACCATCATCGATGGCCAGCCAACCTACAACGTGGA  
 F S T F E L F L T I I D G P A N Y N V D  
 1870 1890 1910  
 CCTGCCCTCATGTACAGCATCACCTATGCTGCCCTTGCCATCATGCCACACTGCTCAT  
 L P F M Y S I T Y A A F A I I A T L L M  
 1930 1950 1970  
 GCTCAACCTCCTCATTGCCATGATGGCGACACTCACTGGCGAGTGGCCATGAGCGGGA  
 L N L L I A M M G D T H W R V A H E R D  
 1990 2010 2030  
 TGAGCTGTGGAGGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCCTCG  
 E L W R A Q I V A T T V M L E R K L P R  
 2050 2070 2090  
 CTGCGCTGTGGCCTCGCTCCGGATCTGGGACGGGAGTATGGCTGGGGACCGCTGGTT  
 C L W P R S G I C G R E Y G L G D R W F  
 2110 2130 2150  
 CCTCGGGGTGGAAGACAGGCAAGATCTCAACCGGCAGGGATCCAACGCTACGCACAGGC  
 L R V E D R Q D L N R Q R I Q R Y A Q A  
 2170 2190 2210  
 CTTCCACACCCGGGGCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGG  
 F H T R G S E D L D K D S V E K L E L G  
 2230 2250 2270  
 CTGTCCCTCAGCCCCACCTGTCCCTTACGCCCTCAGTGTCTCGAAGTACCTCCCG  
 C P F S P H L S L P T P S V S R S T S R  
 2290 2310 2330  
 CAGCAGTGCCAATTGGGAAAGGCTGGCAAGGGACCGCTGAGGAGAGACCTGCGTGGGAT  
 S S A N W E R L R Q G T L R R D L R G I  
 2350 2370 2390  
 AATCAACAGGGTCTGGAGGACGGGAGAGCTGGAAATATCAGATCTGACTGCGTGTCT  
 I N R G L E D G E S W E Y Q I  
 2410 2430 2450  
 CACTTCGCTCCTGGAACTTGCTCTCATTTCCCTGGTGATCAAACAAAAACAAAAACCA  
 2470 2490 2510  
 AACACCCAGAGGTCTCATCTCCAGGCCAGGGAGAAAGAGGAGTAGCATGAACGCCAA  
 2530 2550 2570  
 GGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGCTGGGGCAGA

Fig. 7 / continuation 2

2590	2610	2630
GGAAGCCCAGCCCCAAGCACGGGGCTGGCAGGGCGTGAGGAAC	CTCCTGTGGCCTGCTCA	
2650	2670	2690
TCACCCCTCCGACAGGAGCACTGCATGTCAGAGCACTT	AAAAACAGGCCAGCCTGCTTG	
2710	2730	2750
GGCCCTCGGTCTCCACCCCAGGGTCATAAGT	GGGGAGAGAGCCCTCCCAGGGCACCCAG	
2770	2790	2810
GCAGGTGCAGGGAAAGTGCAGAGCTTGTGGAAAGCGTGTGAGT	GAGGGAGACAGGAACGGC	
2830	2850	2870
TCTGGGGGTGGGAAGTGGGGCTAGGTCTGCCAAC	CCATCTCAATAAAGTCGTTTCG	
2890	2910	
GATCCCTAAAAAAAAAAAAAAAAAAAAAA		

MGLSLPKEKGLIICLWSKFCRWFQRRESWAQSRDEQNLQQKRIWESPLLLAAKDNDVQALNKLKYEDCKVHQRGAMGETALHIA  
ALYDNLEAMVLMEEAPELVFEPMTSELYEGQTAHLIAVVNNQNMNLVRALLARRASV SARATGTA FRRSPRNLIYFGEHPLSFAAC  
VNSEEIVRLLIEHGADIRAQDSLGNNTVLHILILQPNKTFACQMYNLLLSYDRHGDHLQPLDLPVNHQGLTPFKLAGVEGNTVMFQH  
LMQKRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLLELIITTKKREARQILDQTPVKEVSLWKRYGRPYFCLMLGAIYLLYIICFT  
MCCIYRPLKPRTRNNRTSPRNTLLQQKLLQEAYVTPKDDIRLVGELVTVIGAIILLVEVPDIFRMGVTRFFGQTILGGPFHVLI  
TYAFMVLVTMVMRLISASGEVVPMFSALVLGWCNMYFARGFQMLGPFTIMIQKMFQDLMRCWLMMAVVLGFASAFYIIFQTED  
PEELGHFYDYPMALFSTFELFLTIIDGPANYNVDLPPMYSITYAAFIAIATLLMLNLLIAMMGDTHWRAVHERDELWRAQIVATT  
MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRIQRYAQAFHTRGSELDKDSVEKLELGCPFSPLSLPTPSVRST  
SRSSANWERLRLQGTLRRDLRGIIINRGLEDGESWEYQI

Figure 8:

A)

ATGGGTTGTCAGTCCCCAAGGAGAAAGGGCTAATTCTCT  
 M G L S L P K E K G L I L C  
 250 270 290  
 GCCTATGGAGCAAGTTCTGCAGATGGTCCAGAGACGGGAGTCCTGGGCCAGAGCCGAG  
 L W S K F C R W F Q R R E S W A Q S R D  
 310 330 350  
 ATGAGCAGAACCTGCTGCAGCAGAACAGGGATCTGGGAGTCTCCTCTCCTCTAGCTGCCA  
 E Q N L L Q Q K R I W E S P L L A A K  
 370 390 410  
 AAGATAATGATGTCCAGGCCCTGAACAAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACC  
 D N D V Q A L N K L L K Y E D C K V H Q  
 430 450 470  
 AGAGAGGAGCCATGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGG  
 R G A M G E T A L H I A A L Y D N L E A  
 490 510 530  
 CCGCCATGGTGTGATGGAGGCTGCCCGAGCTGGTCTTGAGCCATGACATCTGAGC  
 A M V L M E A A P E L V F E P M T S E L  
 550 570 590  
 TCTATGAGGGTCAGACTGCACTGCACATCGCTGGTGAACCAAGAACATGAAACCTGGTGC  
 Y E G Q T A L H I A V V N Q N M N L V R  
 610 630 650  
 GAGCCCTGCTGCCCGCAGGGCCAGTGTCTGCCAGAGCCACAGGCAGTGCCTTCCGCC  
 A L L A R R A S V S A R A T G T A F R R  
 670 690 710  
 GTAGTCCCTGCAACCTCATCTACTTGGGAGCACCCCTTGTCCCTTGCTGCCTGTGA  
 S P C N L I Y F G E H P L S F A A C V N

Fig. 8 / continua in 1

730	750	770
ACAGTGAGGAGATCGTGGCTGCTCATGGAGCTGACATCCGGGCCAGGACT		
S E E I V R L L I E H G A D I R A Q D S		
790	810	830
CCCTGGGAAACACAGTGTACACATCCTCATCCTCCAGCCAAACAAACCTTGCCTGCC		
L G N T V L H I L I L Q P N K T F A C Q		
850	870	890
AGATGTACAACCTGTTGCTGCTTACGGACAGACATGGGACACCTGCAGCCCTGGACC		
M Y N L L L S Y D R H G D H L Q P L D L		
910	930	950
TCGTGCCAATCACCAAGGGTCTCACCCCTTCAAGCTGGCTGGAGTGGAGGGTAACACTG		
V P N H Q G L T P F K L A G V E G N T V		
970	990	1010
TGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGA		
M F Q H L M Q K R K H T Q W T Y G P L T		
1030	1050	1070
CCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGATGAGCAGTCCCTGCTGG		
S T L Y D L T E I D S S G D E Q S L L E		
1090	1110	1130
AACTTATCATCACCAAGAAGCGGGAGGCTGCCAGATCTGGACCAAGACGCCGGTGA		
L I I T T K K R E A R Q I L D Q T P V K		
1150	1170	1190
AGGAGCTGGTGGCCTAAGTGGAGCGGTACGGCGCCGTACTCTGCAATGCTGGTG		
E L V S L K W K R Y G R P Y F C M L G A		
1210	1230	1250
CCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGC		
I Y L L Y I I C F T N C C I Y R P L K P		
1270	1290	1310
CCAGGACCAATAACCGCACGAGCCCCGGGACAACACCCCTCTACAGCAGAAGCTACTTC		
R T N N R T S P R D N T L L Q Q K L L Q		
1330	1350	1370
AGGAAGCCTACATGACCCCTAACGGACGATATCCGGCTGGTCGGGAGCTGGTACTGTCA		
E A Y M T P K D D I R L V G E L V T V I		
1390	1410	1430
TTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTCAGAAATGGGGTCAC		
G A I I I L L V E V P D I F R M G V T R		
1450	1470	1490
GCTTCTGGACAGACCATCCTGGGGCCCATCCATGTCTCATCACCTATGCC		
F F G Q T I L G G P F H V L I I T Y A F		
1510	1530	1550
TCATGGTGTGGTGGTACCATGGTATGCCCTCATCAGTGCCAGGGAGGTGGTACCCA		
M V L V T M V M R L I S A S G E V V P M		
1570	1590	1610
TGTCTTGCACCGTGTGGTGGCAACGTCTACGTACTTCGCCAGGATTCCAGA		
S F A L V L G W C N V M Y F A R G F Q M		
1630	1650	1670
TGCTAGGCCCTTCACCATCATGATTCAAGAAGATGATTTGGCGACCTGATGCGATTCT		
L G P F T I M I Q K M I F G D L M R F C		
1690	1710	1730
GCTGGCTGATGGCTGGTCATCTGGCTTGGCTCAGCCTCTATATCATCCTCCAGA		
W L M A V V I L G F A S A F Y I I F Q T		
1750	1770	1790
CAGAGGACCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGCCCTGTCAGCACCT		
E D P E E L G H F Y D Y P M A L F S T F		
1810	1830	1850
TCGAGCTGTTCTTACCATCATCGATGCCAGCCAACTACAACGTGGACCTGCCCTCA		
E L F L T I I D G P A N Y N V D L P F M		
1870	1890	1910
TGTACAGCATCACCTATGCTGCCATCATGCCACACTGCTCATGCTCAACCTCC		
Y S I T Y A A F A I I A T L L M L N L L		
1930	1950	1970
TCATGCCATGATGGCGACACTCACTGGCGAGTGGCCATGAGCGGGATGAGCTGTGGA		

Fig. 8 / continu on 2

I	A	M	M	G	D	T	H	W	R	V	A	H	E	R	D	E	L	W	R
1990																			2030
GGGCCCAGATTGTGGCCACCACGGTGTATGGAGCGGAAGCTGCCCTCGCTGCCGTGGC																			
A	Q	I	V	A	T	T	V	M	L	E	R	K	L	P	R	C	L	W	P
2050																			2090
CTCGCTCCGGGATCTGGGACGGGAGTATGGCTGGAGACCCCTGGTCCCTGGGGTGG																			
R	S	G	I	C	G	R	E	Y	G	L	G	D	R	W	F	L	R	V	E
2110																			2150
AAGACAGGAAGATCTAACCGGCAGGGATCCAAACGCTACGCACAGGCCTCCACACCC																			
D	R	Q	D	L	N	R	Q	R	I	Q	R	Y	A	Q	A	F	H	T	R
2170																			2210
GGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGCTGTCCCTCA																			
G	S	E	D	L	D	K	D	S	V	E	K	L	E	L	G	C	P	F	S
2230																			2270
GCCCCCACCTGTCCCTTCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCA																			
P	H	L	S	L	P	M	P	S	V	S	R	S	T	S	R	S	S	A	N
2290																			2330
ATTGGGAAAGGCTCGGCAAGGGACCTGGAGAGACCTGCGTGGGATAATCAACAGGG																			
W	E	R	L	R	Q	G	T	L	R	R	D	L	R	G	I	I	N	R	G
2350																			2390
GTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGA																			
L	E	D	G	E	S	W	E	Y	Q	I	*								

MGLSLPKEKGLLILCLWSKFCRWFORRESWAQSRDEQNLQOKRIWESPLLLAAKDNDVQALNKLKYEDCKVHQRGAMGETALHIA  
 AILYDNLEAAMVLMEAAPELVFPMTSELYEGQTAHLIAVNQNQNMNLVRALLARRASVSARATGTAFRSPCNLIYFGEHPLSFAAC  
 VNSEEIVALLIEHGADIRAQDSLGNTVLHILILQPNKTFAQCQMYNLLLSYDRHGDHQPLDLVPHQGLTPFKLAGVEGNTVMFQH  
 LMQRKRTHTQWTYGPSTLYDLTEIDSSGDEQSLLIELIITKKREARQILDQTPVKEVLWSLKWKRYGRPYFCMLGAIYLLYIICFT  
 MCCCIYRPLKPTNNRTSPRDNTLQQKLLQEAQYMPKDDIRLVEGLVTVIGAIIILVEVPDIFRMGVTRFFQTIILGGPFHVLI  
 TYAFMVLVTMVMRLISASGEVVPMSFALVLGWCNVMYFARGFQMLGPFTIMIQKMIFGDLMRFCWLMAVILGFASAFYIIIFQTED  
 PEELGHFYDYPMALFSTFELFLTIDGPANYNVDPFMYSITYAAFAIIATLILMLNLLIAMGDTHWRVAHERDELWRAQIVATT  
 MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRQIORYAQAFHTRGSELDKDSVEKLELGCPSPHLSLPMPSVSRT  
 SRSSANWERLRQGTLRRDLRGIIINRGLEDGESWEYQI

b)

CAAACCTCACAGCCCTCTCCAAACTGGCTGGGCTGCTGGAGACTCCAAAGGAACCTGTCAGGAAGGGCAGGAGACAGGAGACGGGA  
 CCTCTACAGGGAGACGGTGGGCGCCCTGGGGGGCTGATGTGGCCCAAGGTGAGTCCCTCGAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT  
 GGCCCCCAAGGAGCCGGCCCTACACCCATGGGTTGTCACTGCCCAGGGAAAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT  
 GCAGATGGTTCAGAGCAGGGAGTCCTGGGCCCAGGGCAGATGAGCAGAACCTGCTGAGGAGATCTGGAGGATCTGGAGTCTCC  
 CTCTCTAGCTGCCAAAGATAATGATGTCCAGGGCCCTGAACAAGTTGCTCAAGTATGAGGAGATGCAAGTGCACCAGAGGGAGC  
 CATGGGGAAACAGCGCTACACATAGCAGGCCCTCTATGACAACCTGGAGGCCCATGGTGTGATGGAGGCTGCCCGAGCTGG  
 TCTTTGAGCCCAGACATCTGAGCTATGAGGGTCAGACTGCACTGCACATGCTGTTGTGAACCAGAACATGAACTGGTCTGCA  
 GCCCCGCTTGCCCCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCACCTGCTTCCCGCTAGTCCCCGCAACCTCATCTACTTGG  
 GGAGCAGACCCCTTGTCTTGTCTGCTGTAACAGTGAGGAGATCTGCGGCTGCTCATTGAGCATGGAGCTGACATCCGGCC  
 AGGACTCCCTGGCCCAACAAAACCTTGTCTGCTGCAAGATGTAACACCTGTTGTGCTCTACGACAGAACATGGGACCACCTGCAGCC  
 CCTGGACCTCGTGCCTTCAACCCAGGGCTCAGGCTTCAAGCTGGCTGGAGTGGAGGTAACACTGTGATGTTTCAACCTGA  
 TGCAGAAGCCGAAGCACACCCAGGGACCTATGGACCACTGACCTGACTCTCATGACTCACAGAGATCGACTCCCTCAGGGGAT  
 GAGCAGTCCCTGCTGGAACTTATCATCACCAACCAAGAACGGAGGCTGCCAGATCTGGACAGACGCCGTGAAGGGAGCTGG  
 GAGCCTCAAGTGGAAAGGGTACGGGGGGCGTACTCTGCTGATGCTGGTGCCTATATCTGCTGATCATCTGCTCACCAGT  
 GCTGCATCTACCGCCCCCTCAAGCCAGGACCAATAACCGCACAAGCCCCGGACAACCCCTCTTACAGCAGAACAGTACTTCAG  
 GAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGAGCTGGTACTGTCATGGGCTATCATCTGCTGGTAGA  
 GGTTCCAGACATCTTCAGAATGGGGTCACTCGCTTCTTGGACAGACCCATCTGGGCCCCATTCCATGTCTCATCATCACCT  
 ATGCCCTCATGGTGTGGTACCATGGTGTGCGCTCATATGATTTTGGCAGCTGATGCGATTCTGCTGGCTGATGGCTGTGG  
 TCATCCTGGCTTTGCTTCAAGCTTCTATATCATCTTCCAGACAGAGGACCCAGGGAGCTAGGCCACTCTACGACTACCCCATG  
 GCCCTGTTCACTGGCCTTGCAGCACCTCGAGCTGCTCTTACCATCATCGATGGCCAGCCACTAACAGTGGACCTGCCCTCATGTACAGCAT  
 CACCTATGCTGCCATTGCCATCATGCCACACTGCTCATGCTCAACCTCTCATGCCATGATGGGACACTCACTGGCGAGTGG  
 CCCATGAGGGGGATGAGCTGTGGAGGGCCAGATGTGGCACCACGGTGTGATGCTGGAGCGGAAGCTGCCCTGCTGCC  
 CGCTCCGGCATCTGCGGACGGAGTATGGCTGGGGACCGCTGGTCTGCGGGTGGAGACAGGCAAGATCTCAACCGGCAGCG

C.1

CAAACTCAGGCCCTCCTCCAAACTGGCTGGGGCTGCTGGGGAGACTCCCAGGAAGGACTCGCAGGAAGGCAGGAGACAGGAGACGGGA  
CCTCTACAGGGAGACGGTGGGCCGCCCTGGGGGGCTGATGTCAGGCCCAAGGCTGAGTCCCCTGAGGGCTGGCCCTCGGCCCTCA  
GGCCCCCAAGGAGCCGCCCTACACCCATGGGTTGCACTGCCCAAGGAGAAAGGGTAATTCTCTGCCATGGAGCAAGGTTCT  
GCAGATGGTCCAGAGACGGGAGTCTGGGCCAGAGCCGAGATGAGCAGAACCTGCTGAGCAGAAGAGGATCTGGGAGTCTCCCT  
CTCTTCTAGCTGCCAAAGATAATGATGTCAGGCCCTGACAAGTTGCTCAAGTATGAGGATTGCAAGCTGCCAGAGAGGAGC  
CATGGGGAAACAGCGCTACACATAGCAGGCCCTATGACPACCTGGAGGCCCATGGTCTGATGGAGGCTGCCCGAGCTGG  
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGCTCTGACTGCCATCATGAAACGCCCTGAAATGCCAGGGCTAGAG  
AAGAGGAAGAGATGGGAGCAGCTGGATCCCTGGGATCCTGAAACACCCGAGAGCTCCCTGTTCTCCATCCAGGCTACCCCTGA  
GGGAAAGAGACTGGGGTGCATATGGGAGGGACCCCTGAGGATCTGGGGACAGACCCGTGACTGACAGCTGCTCTGGCCAGG  
TCAGACTGCACATCGCTGTTGAAACAGAACATGAAACCTGGTGGAGGCCCTGCTGCCCGAGGCCAGTGTCTCTGCCA  
GAGCCACAGGCACTGCCCTCCGGCTAGTCCCTGAAACCTCATCTACTTGGGAGCACCCCTTGTCTTGTGCCCTGTGAAC  
AGTGAGGAGATCGTGGCTGCTCATGGAGCATGGAGCTGACATCCGGGCCAGGACTCCCTGGCCCAACAAAACCTTGTGCTGCC  
AGATGTACACCTGTTGCTGCTACGACAGACATGGGACCCACTGAGGCCCTGGACCTCGTGCCCAATCACCAAGGGTCTCAC  
CCTTTCAAGCTGGCTGGAGGTAAACACTGATGTTCTAGCACCTGATGCGAGAAGCGGAAGCACACCCAGTGGACGTATGG  
ACCACTGACCTCGACTCTCATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACC  
AGAAGCGGGAGGCTGCCAGATCTGGACAGAGCCGGTGAAGGAGCTGGTGGCCCTCAAGTGGAAAGCGGTACGGCGGGCGTAC  
TTCTGCAATGCTGGGTCATATATCTGCTGACATCATCTGCTTCAACCATGTCGACATCTACCGCCCCCTCAAGGCCAGGACCA  
TAACCGCAGAGCCCCCGGGAACACCCCTCTACAGCAGAAGCTACTCAGGAAGGCCATACATGACCCCTAAGGAGCATATCCGG  
TGGTCGGGGAGCTGGTGAATGTCATGGGCTATCATCATCTGCTGGTAGAGGTTCCAGACATCTCAGAATGGGGTCACTGCG  
TTCTTGGACAGACCATCTTGGGGCCATTCTCATGTCCTCATCATCACCTATGCTTCATGTTGCTGGTGAACATGGTATGCC  
GCTCATGAGTGGCAGCCCCGGAGGGTGTACCATGCTTGCAGTGGGACTCTGCTGGCTGGCGACCTGTCAGTACTCTGGGG  
TCCAGATGCTAGGCCCTTCACCATCATGATTAGAAGAGATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGGTC  
ATCCGGGCTTTGCTTAGACAGAGGACCCCGAGGAGCTAGGGCAACTTCTACGACTACCCCATGGCCCTGTCAGCACCTTGCA  
GGTCTTACCATCATGATGGCCAGCAACTAACAGTGGACCTGCCCTCATGTCAGCATCACCTATGCTGCCCTTGGCA  
TCCGGCACACTGCTCATGCTCAACCTCTCATGGCATGAGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGG  
AGGGCCCAAGATTGTCGGCACCAGGTGATGCTGGAGGGAGCTGCCCTGCTGCCCTGTCAGGCCATGAGCGGGATCTGGGACGG  
GTATGGCTGGAGACCGCTGGTCTCTGCGGGTGGAGACAGGCAAGATCTCAACGGCAGCGGATCCACCGCTACCCACAGG  
TCCACACCCGGGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGCTGTCCTTCAAGCCCCCACCTGTC  
CCTATGCCCTCAGTGTCTCGAAGTACCTCCCGAGCAGTGCATTGGGAAAGGCTTGGCAAGGGACCCCTGAGGAGAGACCTGCG  
TGGGATAATCAACAGGGGCTGGAGGAGGGAGCTGGAAATATCAGATCTGCTGTTCTCACTGCCCTCTGGAACTT  
GCTCTCATTTCTGGGTCATCAAACAAAACAAAACACCCAGGGCTCATCTCCAGGCCAGGGAGAAAGAGGAGT  
AGCATGAACGCCAAGGAATGTCAGTTGAGAATCACTGCTCCAGGCCATTAACCTCTCAGCTCTGGGGAGAGGAAGGCCAGCC  
CAAGCACGGGCTGGCAGGGCTGAGGAACACTCTCTGTCAGGCCATCACCTTCCAGGGAGCACTGCACTGTCAGAGCACTT  
TAAAAACAGGCCAGGCCCTGCTTGGGGCCCTCGGCTCCACCCAGGGCTATAAGTGGGGAGAGAGGCCCTTCCAGGGCACCAGGCAG  
GTGCAGGGAAAGTGCAGAGCTGTCAGGAGACAGGCTGAGTGCAGGGAGACAGGAACGGCTCTGGGGTGGAAAGTGGGGCTAGGTCTT  
CCAACTCCATCTCAATAAGTCGTTTCCGGATCCCTAaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa

D.)

CAAACCTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACCTGGCTCAGGAAGGCAGGAGACAGGAGACGGGA  
CCTCTACAGGGAGACGGTGGGCCGCCCTGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCCTCAGGGCTCTGGCCTCGGCCCTCA  
GGCCCCCAAGGAGCCGCCCTACACCCCATGGTTTGTCACTGCCCAAGGAGAAAGGCTAATTCTCTGCCATGGAGCAAGTTCT  
GCAGATGGTTCAGAGACGGGAGTCTGGCCAGAGCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCT  
CTCCTCTAGCTGCCAAGATAATGATGTCCAGGCCCTGACAAGTTGCTCAAGTATGAGGATTGCCAAGGTTGCAACCAGAGAGGAGC  
CATGGGGAAACAGCGTACACATAGCAGGCCCTATGACAACCTGGAGGCCATGGCTGATGGAGGCTCCCCGGAGCTGG  
TCTTTGAGCCCATGACATCTGAGCTCATGGGGTCAGACTGCACTGCCACATGCCATGTGTGAACCAAGAACATGAAACCTGGTGCAG  
GCCCTGCTGCCGCCAGGGCCAGTGTCTGCCAGACCCACAGGCAGTCCCTGCCAGTCTCCCGTAGTCCCCGCCACCTCATCTACTTTGG

AAACACAGTGTACACATCCTCATCCTCCAGCCAAACAAAACCTTGCCTGCCAGATGTACAACCTGTTGCTGCTACGACAGAC  
 ATGGGGACCACTGCAAGCCCCCTGGACCTCGTCCAAATCACCAAGGGCTCACCCCTTCAAGCTGGCTGGAGTGGAGGGTAAACACT  
 GTGATGTTCTAGCACCTGATGCAAGCAGGAAAGCACACCCAGTGGACGATGGACCACTGACCTCGACTCTATGACCTCACAGA  
 GATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGAACTTATCATCACCAAGAAGCGGGAGGCTGCCAGATCCTGGACCAGA  
 CGCCGGTCAAGGAGCTGGTGGCTCAAGTGGAGCGGTACCGGCGCCGACTCTGCTGGTGCCTATATCTGCTGTAC  
 ATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGCCCAGGACCAATAACCGACAAGCCCCGGGACAACCCCTT  
 ACAGCAGAAGCTACTTCAGGAAGCCATCGTACCCCTAAGGACGATATCCGGCTGGCGGGAGCTGGTACTGTCATTGGGCTA  
 TCATCATCTGCTGGTGGAGGTTCCAGACATCTCAGAATGGGGCTACTCGCTTCTTGGACAGACCATCCTGGGGGCCATT  
 CATGTCCTCATCATCACCTATGCCTCATGGTGTGGTGCACATGGTATGGGGCTCATCAGTGCACGGGGAGGTGGTACCCAT  
 GTCTTGCACCTGCTGGGGTGGCAACCTGATGCAAGGAGCTAGGCAACTTCTACGACTACCCATGGCCCTGTCAAGCACCTCGAGCTGGTCCITACCAT  
 CATCGATGCCCAAGCAACTACAACGTGGACCTGCCCTCATGTACAGCATCACCTATGCTGCCCTGCCATCATGCCACACTGC  
 TCATGCTCAACCTCCTATTGCCATGATGGCGACACTCAGTGGCAGTGGCCATGAGCGGGATGAGCTGTGGAGGGCCAGAATT  
 GTGGCCACACGGTGTGCTGGAGCGGAAGCTGCCCTGCTGCCCTGCTGGGGATCTGGGAGCGGGAGTATGGCTGG  
 GGACCGCTGGTCCCTGCCGGTGGAGACAGGGCAAGATCTAACCGGCAAGGGATCCACCGCTACGACAGGCCCTCCACACCCGGG  
 GCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAGACTAGAGCTGGGCTGTCCCTCAGCCCCCACCTGTCCTTCTACGCCCTCA  
 GTGTCTGAGAAGTACCTCCGACAGTGCATGGAAATGGGAAAGGCTTCGGCAAGGGACCCCTGAGGAGAGACCTGCGTGGGAATTAACTCAA  
 CAGGGGCTGGAGGAGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCTCAGTCCTGGGCTCCAGGGAGAAAGAGGAGTAGCATGAACGCC  
 CTGGGTGCAAAACAAAAACACCCAGGGCTCATCTCCAGGCTGCATTACTCTCTCAGCTCTGGGAGAGGAAGGCCAGCCAGCACGGG  
 AAGGAATGTACGTTGAGAATCACTGCTCCAGGCTGCATTACTCTCTCAGCTCTGGGAGAGGAAGGCCAGCCAGCACGGG  
 TGGCAGGGCTGAGGAACTCTCTGCTGGCTCATCACCCTCCGACAGGAGCACTGCACTGCAAGAGCACTTAAAAACAGGGC  
 AGCCTGCTGGCCCTCGGTCTCCACCCCAGGGCTATAAGTGGGAGAGGAGGCCCTCCAGGGCACCCAGGGCAGGTGCAGGGAGT  
 GCAGAGCTGTGGAAAGCGTGTGAGTGGAGGAGACAGGAACGGCTCTGGGGTGGGAAGTGGGCTAGGTCTTGCCTACCTCCAT  
 TCAATAAAAGTCGTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAA

e.)

CACACATGGGGCCTCCAGGAGTGCCAGGACCTCGTCTGTTGGCTCTGAATCTATCGTCTCCAAATCGCTGTCCCACAGAAC  
 CATATAACCCACCTCTGTAAATGCCAGGAGCCATGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACACACTGGAGGCC  
 CCATGGTGTGCTGATGGAGGCTGCCCTGGAGCTGGCTTGTGAGGCCATGACATCTGAGCTCTATGGAGGGTGAGGGCCACGGGCTG  
 GGGTGAAGAGCAGGAGTGTGCTGGTATTCAAGTCAGTCTCTGTGATGGATAATTGGGAAAGACACAGGGGATCTGAGCCT  
 CCTACTCTTTSTCTCTCTGCTCCCTCCGCTGTCAGTCCTGACTGCCATCACCTGACGCCCTGCCCCCTGAAATGCCAGGG  
 GCCTAGAGAAGAGGAAGAGATGGCAGCAGCTGGATCCCCCTGGAAATCTGAACACCCAGAGGCTCCCTGTTCTCCATCCCAGGG  
 ACCCTGAGGGAAAGAGACTAGGGTGCATATGGGAGGGACCCCTGCAAGGATCTAGGGGAGACAGACCCGTGACTGACAGCTGTCT  
 CTGGGCCAGGTCAAGACTGCACTGCACATCGCTGTTGTGAAACAGAACATGAACCTGGTGCAGGCCCTGTTGCCCGAGGGCCAGT  
 GTCTCTGCCAGAGCCACAGGCACTGCCCTCCGCGTAGTCCCTGCAACCTCATCTACTTGGGAGCACCCCTTGTCTTGT  
 CTGTTGTAACAGTGAGGAGATGCTGGGCTGCTATTGAGCATGGAGCTGACATCGGGGCCAGGACTCCCTGGATGTACAACCTG  
 TTGCTGTCTTACGACACATGGGACACCTGCAAGGCCCTGGACCTCTGCAACCTGATGCAAGCGGAAGCACACCCAGTGGACGATGGACACTGACCTCGA  
 CTCTCTATGACCTCACAGAGATGACTCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACCAACAGGGAGGCT  
 CGCCAGATCTGGACCAAGGCCGGTGAAGGAGCTGGTGAAGGACTGCTCAAGTGGAGCGGTACGGGCCAGGACTTCTGCACTGCTGG  
 TGCCATATCTGCTGTACATCATCTGCTTACCATGTGCTGCATCTACCGGCCCTCAAGGCCAGGACCAATAACCGCACAGCC  
 CCCGGACAAACACCCCTTACAGCAGAAGCTACTCAGGAAGCCTACATGACCCCTAACGGAGCATACTGGCTGTCGGGAGCTG  
 GTGACTGTCATTGGGCTATCATCATCTGCTGTTAGGGATTCAGACATCTCAGAATGGGGCTACTCGCTTCTTGGACAGAC  
 CATCCCTGGGGCCCATCCATGCTCATCATCACCTATGCCCTCATGGTGTGGTACCATGGTATGCGGCTCATCAGTGC  
 GCGGGGAGGTGGTACCCATGTCCTTGCACTCGCTGGCTGGTCAACGCTCATGTAATTGCCCTGGGATTCAGATGCTAGGC  
 CCCCTCACCATCATGATTCAGAAGAGATGATTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTTGTCATCCTGGGTTGC  
 TTCAGCCTCTATATCATCTTCCAGACAGGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCATGCCCTGTTGCA  
 TCGAGCTGTCCTTACCATCATGATGGCCAGGCAACTAACAGTGGACCTGCCCTCATGTAACGATCACCTATGCTGCTT  
 GCCATCATGCCACACTGCTCATGCTCACCTCTCATTGCATGATGGCGACACTCACTGGCAGTGGCCCATGAGCGGGATGA  
 GCTGTGGAGGGCCCAAGATTGTCGGGACCCACGGTGTGCTGGAGCGGAAGCTGCCCTGCTGGCTCGCTGGGATCTGCG  
 GACGGGAGTATGGCCTGGAGACCGCTGGTCTCTGCCGGGTGGAAGACAGGCAAGATCTAACCGGCCAGGGATCAACGCTACGCA  
 CAGGCCCTCCACACCCGGCTCTGAGGATTGGACAAAGACTCAGTGGAAAAGACTAGAGCTGGCTGTCCTCAGGCCACCT  
 GTCCCTTCTATGCCCTCATGTCAGTGTGAAAGTACTCTCCGAGCAGCTGCAATTGGGAAAGGCTTCGGCAAGGGACCCCTGAGGAGAG  
 ACCTGCGTGGGATAATCAACAGGGGCTGGAGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTCTCAGTCCTGCTT  
 GGAACCTTGTCTCATTTCTGGGTGCATCAAACAAAACAAAACACCCAGGGCTCATCTCCAGGCCAGGGAGAAA  
 GAGGAGTAGCATGAACGCCAAGGAATGTACGTTGAGAATCAGTCCAGGCCCTGCATTACTCCTCAGCTGTCCTGGGGCAGAGGAAG  
 CCCAGCCCAAGCACGGGCTGGCAGGGCTGAGGAACCTCTCTGTGGCTCATCACCCCTCCGACAGGAGCAGTGCATGTCAG  
 AGCACTTAAAAACAGGCCAGGCCCTGGTCTGCCACCCAGGGCTATAAGTGGGAGAGGAGGCCCTCCAGGGCACC

Fig. 8 / continuation 5

CAGGCAGGTGCAGGGAAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGTGGGAAGTGGGCTA  
GGTCTGCCAACTCCATCTTCAATAAAGTCGTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAA

Figure 9:

A.

10	30	50
CGGGGCCCTGGGCTGCAGGAGGTTGGCGGCCGGCAGCATGGTGGTGCCGGAGAAGG		
		M V V P E K E
70	90	110
AGCAGAGCTGGATCCCAAGATCTTCAAGAAGAACCTGCACGACGTTCATAGTTGACT		
Q S W I P K I F K K K T C T T F I V D S		
130	150	170
CCACAGATCCGGGAGGGACCTTGTGCCAGTGTGGCGCCCCCGGACCGCCCACCCCGCAG		
T D P G G T L C Q C G R P R T A H P A V		
190	210	230
TGGCCATGGAGGATGCCTCGGGGAGCCGTGGTGGACCGTGTGGGACAGCGATGCACACA		
A M E D A F G A A V V T V W D S D A H T		
250	270	290
CCACGGAGAAGCCCACCGATGCCTACGGAGAGCTGGACTTCACGGGGCCGGCAAGC		
T E K P T D A Y G E L D F T G A G R K H		
310	330	350
ACAGCAATTCCCTCCGGCTCTGACCGAACGGATCCAGCTGCAGTTATAGTCTGGTCA		
S N F L R L S D R T D P A A V Y S L V T		
370	390	410
CACGCACATGGGGCTCCCGTCCCCGAACCTGGTGGTGTCACTGGCTGGGGGATGGGG		
R T W G F R A P N L V V S V L G G S G G		
430	450	470
GCCCGTCCTCCAGACCTGGCTGCAGGACCTGCTGCGTCGGGCTGGTGCAGGGCTGCC		
P V L Q T W L Q D L L R R G L V R A A Q		
490	510	530
AGAGCACAGGAGCCTGGATTGTCACTGGGGCTGCACACGGCATCGGCCGGATGTTG		
S T G A W I V T G G L H T G I G R H V G		
550	570	590
GTGTGGCTGTACGGGACCATCAGATGCCAGCAGCACTGGGGCACCAAGGTGGTGGCCATGG		
V A V R D H Q M A S T G G T K V V A M G		
610	630	650
GTGTGGCCCCCTGGGTGTGGTCCGGAATAGAGACACCCCTCATCAACCCAAGGGCTCGT		
V A P W G V V R N R D T L I N P K G S F		
670	690	710
TCCCTGCGAGGTACCGGTGGCGCGGTGACCCGGAGGACGGGGTCCAGTTCCCTGGACT		
P A R Y R W R G D P E D G V Q F P L D Y		
730	750	770
ACAACTACTCGCCCTCTTCTGGTGACGACGGCACACACGGCTGCCCTGGGGGGCGAGA		
N Y S A F F L V D D G T H G C L G G E N		
790	810	830
ACCGCTTCCGCTTGCCTGGAGTCCTACATCTCACAGCAGAACGGCTGGAGGGAGGA		
R F R L R L E S Y I S Q Q K T G V G G T		
850	870	890
CTGGAATTGACATCCCTGCTCTGCTCCTGATTGATGGTGTGAGAGAACATGTTGACGC		
G I D I P V L L L I D G D E K M L T R		
910	930	950
GAATAGAGAACGCCACCCAGGCTCAGCTCCCATGTCCTCGTGGCTGGCTCAGGGGGAG		
I E N A T Q A Q L P C L L V A G S G G A		
970	990	1010
CTGCGGACTGCCCTGGCGGAGACCCCTGGAAGAACACTCTGGCCCCAGGGAGTGGGGAGCCA		
A D C L A E T L E D T L A P G S G G A R		
1030	1050	1070
GGCAAGGCGAAGCCCGAGATCGAATCAGGCCTTCTTCCAAAGGGGACCTTGAGGTCC		

Fig. 9 / continu: n 1

Q G E A R D R I R R F F P K G D L E V L  
 1090 1110 1130  
 TGCAGGCCAGGTGGAGAGGATTATGACCCCGAAGGAGCTCCTGACAGTCTATTCTCTG  
 Q A Q V E R I M T R K E L L T V Y S S E  
 1150 1170 1190  
 AGGATGGGTCTGAGGAATTGAGACCATAGTTTGAAGGCCCTGTGAAGGCCGTGGGA  
 D G S E E F E T I V L K A L V K A C G S  
 1210 1230 1250  
 GCTCGGAGGCCTCAGCCTACCTGGATGAGCTGCGTTGGCTGCGCTTGGAACCGCGTGG  
 S E A S A Y L D E L R L A V A W N R V D  
 1270 1290 1310  
 ACATTGCCAGAGTGAACCTTTCGGGGGACATCCAATGGCGGTCTTCCATCTCGAAG  
 I A Q S E L F R G D I Q W R S F H L E A  
 1330 1350 1370  
 CTTCCCTCATGGACGCCCTGCTGAATGACCGCCTGAGTTCGTGCCTGCTCATTTCCC  
 S L M D A L L N D R P E F V R L L I S H  
 1390 1410 1430  
 ACGGCCTCAGCCTGGCCACTTCCGTACCCGATGCGCCTGGCCAACCTACAGCGCG  
 G L S L G R F L T P M R L A Q L Y S A A  
 1450 1470 1490  
 CGCCCTCCAACTCGCTCATCCGCAACCTTTGGACCAAGGCCTCCACAGCGCAGGCACCA  
 P S N S L I R N L L D Q A S H S A G T K  
 1510 1530 1550  
 AAGCCCCAGCCCTAAAAGGGGGAGCTGCGGAGCTCCGGCCCCCTGACGTGGGCATGTGC  
 A P A L K G G A A E L R P P D V G H V L  
 1570 1590 1610  
 TGAGGATGCTGCTGGGGAGATGTGCGCGCCGAGGTACCCCTCCGGGGGCCTGGGACC  
 R M L L G K M C A P R Y P S G G A W D P  
 1630 1650 1670  
 CTCACCCAGGCCAGGGCTCGGGAGAGCATGTATCTGCTCTCGAACAGGCCACCTCGC  
 H P G Q G F G E S M Y L L S D K A T S P  
 1690 1710 1730  
 CGCTCTCGCTGGATGCTGGCTCGGGCAGGGCCCTGGAGCGACCTGCTTCTTGGCAC  
 L S L D A G L G Q A P W S D L L W A L  
 1750 1770 1790  
 TGTTGCTGAACAGGGCACAGATGGCATGTACTCTGGAGATGGTTCCAATGCAGTT  
 L L N R A Q M A M Y F W E M G S N A V S  
 1810 1830 1850  
 CCTCAGCTTGGGCTGTTGCTGCTCCGGGTGATGGCACGCCCTGGAGGCCTGACGCTG  
 S A L G A C L L L R V M A R L E P D A E  
 1870 1890 1910  
 AGGAGGCAGCACGGAGGAAAGACCTGGCTCAAGTTGAGGGATGGCGTTGACCTCT  
 E A A R R K D L A F K F E G M G V D L F  
 1930 1950 1970  
 TTGGCGAGTGCTATCGCAGCAGTGAGGTGAGGGCTGCCGCCTCCTCCGTGCTGCC  
 G E C Y R S S E V R A A R L L L R R C P  
 1990 2010 2030  
 CGCTCTGGGGGATGCCACTGCTCCAGCTGGCATGCAAGCTGACGCCGTGCCCTCT  
 L W G D A T C L Q L A M Q A D A R A F F  
 2050 2070 2090  
 TTGCCAGGATGGGTACAGTCTCTGCTGACACAGAAGTGGTGGGAGATATGCCAGCA  
 A Q D G V Q S L L T Q K W W G D M A S T  
 2110 2130 2150  
 CTACACCCATCTGGCCCTGGTTCTGCCCTCTTGCCTCCACTCATCACACCCGCC  
 T P I W A L V L A F F C P P L I Y T R L  
 2170 2190 2210  
 TCATCACCTTCAGGAAATCAGAAGAGGAGCCACACGGGAGGAGCTAGAGTTGACATGG  
 I T F R K S E E E P T R E E L E F D M D  
 2230 2250 2270  
 ATAGTGTCAATTAAATGGGAAGGGCCTGCGGACGGCGAACCGCCGAGAAGACGCC  
 S V I N G E G P V G T A D P A E K T P L  
 2290 2310 2330

Fig. 9 / continuation 2

TGGGGGTCCCGGCCAGTCGGCCGTCGGGTTGCTGCGGGGCCGCTGCGGGGGCG  
 G V P R Q S G R P G C C C G G R C G G R R R  
 2350 2370 2390  
 GGTGCCTACGCCGCTGGTCCACTTCCTGGGGCGTGCCTGGTACCATCTTCATGGCAACG  
 C L R R W F H F W G V P V T I F M G N V  
 2410 2430 2450  
 TGGTCAGCTACCTGCTGTTCTGCTGCTGCTTCTCGCGGGTGCCTGCTCGTGGATTCCAGC  
 V S Y L L F L L L F S R V L L V D F Q P  
 2470 2490 2510  
 CGCGCCGCCGGCTCCCTGGAGCTGCTGCTATTCCTGGCTTACGCTGCTGTGCG  
 A P P G S L E L L L Y F W A F T L L C E  
 2530 2550 2570  
 AGGAACCTGCCAGGGCTGAGCGAGGGGGCAGCCTCGCCAGCGGGGGGGGG  
 E L R Q G L S G G G G S L A S G G P G P  
 2590 2610 2630  
 CTGGCCATGCCCTACTGAGCCAGCGCTGCCCTCTACCTGCCGACAGCTGGAACAGT  
 G H A S L S Q R L R L Y L A D S W N Q C  
 2650 2670 2690  
 GCGACCTAGTGGCTCTCACCTGCTTCCCTGGCGTGGCTGCCGCTGACCCCGGGTT  
 D L V A L T C F L L G V G C R L T P G L  
 2710 2730 2750  
 TGTACCACCTGGCCGACTGCTCTGCATCGACTTCATGGTTTACGGTGCCTG  
 Y H L G R T V L C I D F M V F T V R L L  
 2770 2790 2810  
 TTCACATCTCACGGTCAACAAACAGCTGGGGCCCAAGATCGTCATCGTGAGCAAGATGA  
 H I F T V N K Q L G P K I V I V S K M M  
 2830 2850 2870  
 TGAAGGACGTGTTCTCTCCTCTCGGCGTGTGGCTGGTAGCCTATGGCGTGG  
 K D V F F F L F L G V W L V A Y G V A  
 2890 2910 2930  
 CCACGGAGGGGCTCTGAGGCCACGGACAGTGACTTCCCAAGTATCCTGCCCGCGTCT  
 T E G L L R P R D S D F P S I L R R V F  
 2950 2970 2990  
 TCTACCGTCCCTACCTGCAGATCTCGGGCAGATTCCCCAGGAGGACATGGACGTGGCCC  
 Y R P Y L Q I F G Q I P Q E D M D V A L  
 3010 3030 3050  
 TCATGGAGCACAGCAACTGCTCGTGGAGCCGGCTCTGGCACACCCCTCTGGGGCCC  
 M E H S N C S S E P G F W A H P P G A Q  
 3070 3090 3110  
 AGGCAGGGCACCTGCGTCTCCAGTATGCCAAGTGGCTGGTGGCTGCTCCTCGTCATCT  
 A G T C V S Q Y A N W L V V L L L V I F  
 3130 3150 3170  
 TCCCTGCTCGTGGCAACATCCTGCTGGTCAACTGCTCATGCCATGTTACACAT  
 L L V A N I L L V N L L I A M F S Y T F  
 3190 3210 3230  
 TCGGCAAAGTACAGGGCAACAGCGATCTACTGGAAAGCGCAGCGTTACCGCTCATCC  
 G K V Q G N S D L Y W K A Q R Y R L I R  
 3250 3270 3290  
 GGGAAATTCCACTCTGGCCCGCGCTGGCCCCGCCCTTATGTCATCTCCACTGCGCC  
 E F H S R P A L A P P F I V I S H L R L  
 3310 3330 3350  
 TCCTGCTCAGGCAATTGTCAGGCAGCCCGGAGCCCCAGCCGTCTCCCGGCCCTCG  
 L L R Q L C R R P R S P Q P S S P A L E  
 3370 3390 3410  
 AGCATTCCGGTTACCTTCTAAGGAAGCGAGCGGAAGCTGCTAACGTGGAAATCGG  
 H F R V Y L S K E A E R K L L T W E S V  
 3430 3450 3470  
 TGCATAAGGAGAACTTCTGCTGGCACCGCTAGGGACAAGCGGGAGAGCGACTCCGAGC  
 H K E N F L L A R A R D K R E S D S E R  
 3490 3510 3530  
 GTCTGAAGCGCACGTCCCAGAAGGTGGACTTGGCACTGAAACAGCTGGGACACATCCGCG  
 L K R T S Q K V D L A L K Q L G H I R E

Fig. 9 / continua 13

3550	3570	3590
AGTACGAACAGCGCCTGAAAGTGCCTGGAGCGGGAGGTCCAGCAGTGTAGCCCGTCTGG		
Y E Q R L K V L E R E V Q Q C S R V L G		
3610	3630	3650
GGTGGCTGGCCGAGGCCCTGAGCCGCTCTGCCCTGCTGCCCTCAGGTGGGCCACCC		
W V A E A L S R S A L L P P G G P P P P		
3670	3690	3710
CTGACCTGCCCTGGGTCCAAAGACTGAGCCCTGCTGGGGACTTCAGGAGAAGCCCCAC		
D L P G S K D *		
3730	3750	3770
AGGGGATTTGCTCCTAGAGTAAGGCTCATCTGGCCCTCGGCCCGCACCTGGTGGCCT		
3790	3810	3830
TGTCCTTGAGGTGAGCCCCATGTCCATCTGGGCCACTGTCAAGGACCACCTTGGAGTGT		
3850	3870	3890
CATCCTTACAAACCACAGCATGCCCGCTCCTCCAGAACCAAGTCCCAGCCTGGAGGAT		
3910	3930	3950
CAAGGCCTGGATCCCGGGCGTTATCCATCTGGAGGCTGCAGGGCCTTGGGTAACAGG		
3970	3990	4010
GACCACAGACCCCTCACCACTCACAGATTCCCTCACACTGGGAAATAAGCCATTCAGA		
4030		
GGAAAAA.....		

MVVEKEQSWIPIKIFKKKCTTTFIVDSTDGGTLCCQGRPRTAHPAVAMEDAFGAAVVTWVDSDAHTTEKPTDAYELDFTGAGRKH  
 SNFLRLSDRTDPAAVSLSVTRTRWGFRAPNVLVSVLGGSGPVLQTLQDILLRGLVRAAQSTGAWIVTGLHTGIGRHGVAVRDH  
 QMASTGGTKVVMGVAPWGVRNRDTLIPKGSFPARYRWRGDPEDGVQFPLDYNYSAFFLVDGTHGCLGGENRFRLRLESYISQ  
 QKTVGGTGIIDIPVLLLIDGDEKMLTRIENATQAHVPCLLVAGSRLGLMPGGTLEAHLAQDGDHKANQSTNQLLPKDLSLQFVE  
 SIDRKTILQSYSERLAVANRVDIAQSELFRGDIQWRSFHLEASLMDALLNDPFEVRLLISHGLSLGHFLTPMRLAQLYSAAPSNS  
 LIRNLLDQASHSAGTKAPALKGGAAEELRPPDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGFGEQMYLLSDKATSPLSLDAGLGQ  
 FWSDLLLWALLNRAQMAMYFWEMGSNAVSSALGACLLLRVMARLEPDAAEARRKDLAFKFEGMGVDLFGECEYRSSEVRAARLL  
 RRCPLWGDATCLQLAMQADARAFFAQDGVQSLLTQKWWGDMASTPTIWLVLIAFFCPPLIYTRLITFRKSEEEPTREEELFDMDSV  
 INGEGPVGTADEPAEKTPLGVPRQSGRPGCCGGRGCRRLRWFHWGVPTIFMGNVSYLLFLLLFSRVLLVDFQFAPPGSLEL  
 LLYFWAFTLLCEELRQGLSGGGGSIASGGPGPGHASLSQRRLYLADESWNQCDLVALTCFLVGVCRLTPGLYHLGRTVLCIDFMV  
 FTVRLLHIETVVKQLGPKIVIVSKMMKDVFVFLFLGVWLAVGVATEGLLRPRDSDFPSILRRVFYRPLQIFGQIPOEDMDVAL  
 MEHSNCSSPEGFWAHEPPGAQAGTCVSQYANWLVLLVFLVANILLVNLLIAMFSYTFGKVQGNSDLWKAQYRLIREFHSRP  
 ALAPPFIVISHRLLLRQLCRRPRSPQPSPALEHFRVYLSKEAERKLLTWESVHKENFLARARDKRESDSERLKRTSQKVDLAL  
 KQLGHIERYEQLKVLEREVQQCSRVLGVVAEALSRALLPPGGPPPDLPGSKD

B.)

10	30	50
ATCCAATGGCGGTCTTCCATCTCGAAGCTCCCTCATGGACGCCCTGCTGAATGACCGG		
70	90	110
CCTGAGTTCTGCGCTTGCTCATTTCCCACGGCCTCAGCCTGGCCACTTCCCTGACCCCG		
130	150	170
ATGCGCCTGGCCCAACTCTACAGCGCCGCCCTCCAACCTCGCTCATCGCAACCTTTG		
190	210	230
GACCAGGCGTCCCACAGCGCAGGCACCAAAGCCCCAGCCCTAAAAGGGGGAGCTGCGGAG		
250	270	290
CTCCGGCCCCCTGACGTGGGCATGTGCTGAGGATGCTGCTGGGAAGATGTGCGCGCCG		
310	330	350
AGATGTATCTGCTCTCGGACAAGGCCACCTCGCCGCTCTGCTGGATGCTGGCCTGGC		
M Y L L S D K A T S P L S L D A G L G Q		
370	390	410
AGGCCCTGGAGCGACCTGCTTCTTGGCACTGTTGCTGAACAGGGCACAGATGGCCA		
A P W S D L L W A L L L N R A Q M A M		
430	450	470
TGTACTTCTGGAGATGGGTTCCAATGCACTTCCAGCTTGGGCCTGTTGCTGC		
Y F W E M G S N A V S S A L G A C L L L		

Fig. 9 / continua 14

490	510	530
TCCGGGTGATGGCACGCCCTGGAGCCTGACGCTGAGGAGGCAGCACGGAGGAAAGACCTGG		
R V M A R L E P D A E E A A R R K D L A		
550	570	590
CGTTCAAGTTGAGGGATGGCCCTGACCTCTTGGCGAGTGCATGCCACCACTGAGG		
F K F E G M G V D L F G E C Y R S S E V		
610	630	650
TGAGGGCTGCCGCCCTCCCTCCGCTGCCGCTCTGGGGGATGCCACTTGCCTCC		
R A A R L L L R R C P L W G D A T C L Q		
670	690	710
AGCTGGCCATGCAAGCTGACGCCGTGCCCTCTTGCCCAGGATGGGTACAGTCTCTGC		
L A M Q A D A R A F F A Q D G V Q S L L		
730	750	770
TGACACAGAAGTGGTGGGAGATATGCCAGCACTACACCCATCTGGGCCCTGGTCTCG		
T Q K W W G D M A S T T P I W A L V L A		
790	810	830
CCTCTTTGCCCTCCACTCATCACACCCGCCCTCATCACCTTCAGGAAATCAGAAGAGG		
F F C P P L I Y T R L I T F R K S E E E		
850	870	890
AGCCCACACGGGAGGAGCTAGAGTTGACATGGATAGTGTCAATTAAATGGGAAGGGCCTG		
P T R E E L E F D M D S V I N G E G P V		
910	930	950
TCGGGACGGGGGACCCAGCCAGCGAGAAAGACGCCGTGGGGTCCCGCGCCAGTCGGGCCGTC		
G T A D P A E K T P L G V P R Q S G R P		
970	990	1010
CGGGTTGCTGCCGGGCGCTGCCGGGGGGCGCCGGCTACGCCGCTGGTCCACTTCT		
G C C G G R C G G R R C L R R W F H F W		
1030	1050	1070
GGGGCGTGCCGGTGACCATCTCATGGCAACGTGGTCAGCTACCTGCTGTTCCGTG		
G V P V T I F M G N V V S Y L L F L L L		
1090	1110	1130
TTTCTCGCGGGTGCTGCTCGTGGATTCCAGCCGGCGCCGGCTCCCTGGAGCTGC		
F S R V L L V D F Q P A P P G S L E L L		
1150	1170	1190
TGCTCTATTCTGGCTTACGCTGCTGTGCAGGAACGTGCCAGGGCTGAGCGGAG		
L Y F W A F T L L C E E L R Q G L S G G		
1210	1230	1250
GGGGGGCAGCCTGCCAGCGGGGCCCGGGCTGGCATGCCACTGAGCCAGCCC		
G G S L A S G G P G P G H A S L S Q R L		
1270	1290	1310
TGCGCCTCTACCTCGCCAGAGCTGGAACAGTGCACCTAGTGGCTCTCACCTGCTTCC		
R L Y L A D S W N Q C D L V A L T C F L		
1330	1350	1370
TCCGGCGTGGCTGCCGGCTGACCCGGTTGTACCACTGGCCGACTGTCTCT		
L G V G C R L T P G L Y H L G R T V L C		
1390	1410	1430
GCATCGACTTCACTGGTTTACGGTGGCTGCTCACATCTCACGGTCAACAAACAGC		
I D F M V F T V R L L H I F T V N K Q L		
1450	1470	1490
TGGGGCCAAGATCGTCATCGTGAGCAAGATGATGAAGGACGTGTTCTTCTTCTTCT		
G P K I V I V S K M M K D V F F F L F F		
1510	1530	1550
TCCCTGGCGTGTGGCTGGTAGCCTATGGCGTGGCACGGAGGGCTCCTGAGGCCACGG		
L G V W L V A Y G V A T E G L L R P R D		
1570	1590	1610
ACAGTGACTTCCCAAGTATCTGCGCCGCTTCTACCGTCCCTACCTGCAGATCTCG		
S D F P S I L R R V F Y R P Y L Q I F G		
1630	1650	1670
GGCAGATTCCCCAGGAGGACATGGACGTGGCCCTCATGGAGCACAGCAACTGCTCGTGG		
Q I P Q E D M D V A L M E H S N C S S E		
1690	1710	1730
AGCCCGGCTTCTGGGACACCCCTCTGGGCCAGGGCAGCTGCGTCTCCCACTATG		

Fig. 9 / continuation 5

P	G	F	W	A	H	P	P	G	A	Q	A	G	T	C	V	S	Q	Y	A				
1750								1770											1790				
CCA	ACT	GGC	TGG	TGG	TG	C	T	C	C	T	C	T	G	C	C	A	A	C	A	T	C	T	G
N	W	L	V	V	L	L	V	I	F	L	L	V	A	N	I	L	L	V					
1810								1830											1850				
TCA	ACT	TG	C	T	A	T	G	C	A	T	C	G	G	A	A	G	T	A	C	A	G	G	A
N	L	L	I	A	M	F	S	Y	T	F	G	K	V	Q	G	N	S	D	L				
1870								1890											1910				
TCT	ACT	TG	G	A	G	G	C	G	C	T	A	C	G	C	C	C	G	C	G	T	G	G	C
Y	W	K	A	Q	R	Y	R	L	I	R	E	F	H	S	R	P	A	L	A				
1930								1950											1970				
CCCC	GCC	C	T	T	T	A	C	G	T	C	C	G	C	T	T	T	C	A	G	G	C	A	C
P	P	F	I	V	I	S	H	L	R	L	L	R	Q	L	C	R	R	P					
1990								2010											2030				
CCC	G	A	G	C	C	C	C	A	G	C	T	C	G	A	T	T	C	A	T	T	C	A	G
R	S	P	Q	P	S	S	P	A	L	E	H	F	R	V	Y	L	S	K	E				
2050								2070											2090				
AAG	CCG	A	G	G	A	G	C	T	G	C	A	T	T	C	T	G	C	A	C	T	G	G	A
A	E	R	K	L	L	T	W	E	S	V	H	K	E	N	F	L	L	A	R				
2110								2130											2150				
GCG	C	T	A	G	G	A	C	G	G	A	G	C	G	C	A	G	T	C	C	A	G	G	T
A	R	D	K	R	E	S	D	S	E	R	L	K	R	T	S	Q	K	V	D				
2170								2190											2210				
ACT	TG	G	C	A	T	G	T	G	G	A	C	A	T	G	C	C	T	G	A	A	G	T	G
L	A	L	K	Q	L	G	H	I	R	E	Y	E	Q	R	L	K	V	L	E				
2230								2250											2270				
AGC	GGG	A	G	G	T	C	A	G	T	G	A	G	C	G	C	G	A	G	C	C	G	T	G
R	E	V	Q	Q	C	S	R	V	L	G	W	V	A	E	A	L	S	R	S				
2290								2310											2330				
CTG	C	T	G	C	T	G	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	G
A	L	L	P	P	G	G	P	P	P	D	L	P	G	S	K	D	*						
2350								2370											2390				
CCCT	G	T	G	G	G	A	T	C	A	G	G	A	G	C	C	C	C	T	A	G	T	A	G
2410								2430											2450				
CAT	T	GG	G	C	T	G	C	C	C	C	T	G	C	T	G	G	T	C	C	T	A	G	T
2470								2490											2510				
CTGG	G	C	A	T	G	T	C	A	G	G	C	C	T	T	A	C	A	C	G	C	T	G	C
2530								2550											2570				
CTCC	T	C	C	A	G	A	C	A	G	T	C	C	T	G	G	A	T	C	C	G	G	G	T
2590								2610											2630				
ATCT	GG	A	G	G	T	G	C	A	GG	G	T	C	T	G	G	A	C	C	C	T	A	C	A
2650								2670											2690				
TTCC	T	C	A	C	T	G	GG	A	A	T	A	A	G	C	T	G	A	A	A	A	A	A	A

MYLLSDKATSPLSLDAGLGQAPWSLLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRLVMARLEPDAEEAARRKDLAFKFEGM  
 GVDLFGEYCYSSEVRAARLLLRCPLWGDATCLQLAMQADARAFFAQDGQVSLLTQKWWGDMASTTPIWALVLAFFCPPLIYTRLI  
 TFRKSEEPTREELEFDMDSVINGEGPVGTDADPAEKTPLGVPRQSGRPGCCGGRGRCGRRCLRRWFHFWGPVTIIFMGNVVSYLLFL  
 LLFSRVLLVDFQPAPPGSLELLLYFWAFTLLCEELRQGLSGGGSLASGGPGPGHASLSQRRLRLYIADSWNQCDLVALTCFLVG  
 CRLTPGLYHLGRTVLCIDFMVFTVRLLHIFTVNKQLGPKIVIVSKMMKDVFLLFLGVWLAVGVATEGLLRPRDSDFPSILRRV  
 FYRPYLQIFGQIPQEDMDVALMEHSNCSEPGFWAHPPGAQAGTCVSQYANLVVLLLVIFLLVANILLVNLLIAMFSYTFGVQG  
 NSDLYWKAQRYRLIREFHSRPALAPPFIVSHRLLLRQLCRRPRSPQSPALEHFRVYLSKEAERKLLTWESVHKENFLRAR  
 DKRESDSERLKRTSQKVDLALKQLGHIREYEQRLKVLEREVQOCRSVVLGVVAEALSRSAALLPPGGPPPDLPGSKD

10 30 50  
 ATTAAAAGTTATAAAACAGTGGCTGGATGGTGGAGGATGCAGGTGGACAGAACGCTGG  
 M V G G C R W T E D V E  
 70 90 110  
 AGCCTGCAGAAGTAAAGGAAAAGATGTCCTTCGGCAGCCAGGCTCAGCATGAGGAACA  
 P A E V K E K M S F R A A R L S M R N R  
 130 150 170  
 GAAGGAATGACACTCTGGACAGCACC CGGACCCCTGTACTCCAGCGCGTCGGAGCACAG  
 R N D T L D S T R T L Y S S A S R S T D  
 190 210 230  
 ACTTGTCTTACAGTGAAGCGCCAGCTCTACGCTGCCTCAGGACACAGACGTGCCAA  
 L S Y S E S A S F Y A A F R T Q T C P I  
 250 270 290  
 TCATGGCTTCTGGGACTTGGTGAATTATTCAAGCAAATTAAAGAAACGAGAATGTG  
 M A S W D L V N F I Q A N F K K R E C V  
 310 330 350  
 TCTTCTTACCAAAGATTCCAAGGCCACGGAGAATGTGTGCAAGTGTGGCTATGCCAGA  
 F F T K D S K A T E N V C K C G Y A Q S  
 370 390 410  
 GCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAAC TACAAGAACACA  
 Q H M E G T Q I N Q S E K W N Y K K H T  
 430 450 470  
 CCAAGGAATTCTACCGACGCCCTGGGATATTCAAGTTGAGACACTGGGAAGAAAG  
 K E F P T D A F G D I Q F E T L G K K G  
 490 510 530  
 GGAAGTATATACGTCTGTCCTGCGACACGGACGCCGAAATCCTTACGAGCTGCTGACCC  
 K Y I R L S C D T D A E I L Y E L L T Q  
 550 570 590  
 AGCACTGGCACCTGAAAACACCCAACCTGGTCAATTCTGTGACCGGGGGCGCAAGAACT  
 H W H L K T P N L V I S V T G G A K N F  
 610 630 650  
 TCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTACATCGCGCAGTCCA  
 A L K P R M R K I F S R L I Y I A Q S K  
 670 690 710  
 AAGGTGCTGGATTCTACGGAGGGACCCATTATGGCTGATGAAGTACATGGGGAGG  
 G A W I L T G G T H Y G L M K Y I G E V  
 730 750 770  
 TGGTGAGAGATAACACCATCAGCAGGAGTTCAAGAGGAATATTGTGGCCATTGGCATAG  
 V R D N T I S R S S E E N I V A I G I A  
 790 810 830  
 CAGCTTGGGCATGGCTCCAACCAGGACACCCCTCATCAGGAATTGCGATGCTGAGGGCT  
 A W G M V S N R D T L I R N C D A E G Y  
 850 870 890  
 ATTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTGTATATCCTGGACA  
 F L A Q Y L M D D F T R D P L Y I L D N  
 910 930 950  
 ACAACCCACACACATTGCTCGTGGACAATGGCTGTCAAGACATCCACTGTGCAAG  
 N H T H L L L V D N G C H G H P T V E A  
 970 990 1010  
 CAAAGCTCCGGAATCAGCTAGAGAAGTATATCTGAGCGCACTATTCAAGATTCCA  
 K L R N Q L E K Y I S E R T I Q D S N Y  
 1030 1050 1070  
 ATGGTGGCAAGATCCCCATTGTGTGTTTGCCTGGAGGAGGTGGAAAAGAGACTTTGAAG  
 G G K I P I V C F A Q G G G K E T L K A  
 1090 1110 1130  
 CCATCAATACCTCCATCAAAATAAAATTCTGTGTGGTGGAGGATGCCCTGACATCTGCGCTCA  
 I N T S I K N K I P C V V V E G S G Q I  
 1150 1170 1190  
 TCGCTGATGTGATCGCTAGCCTGGTGGAGGATGCCCTGACATCTGCGCTCA  
 A D V I A S L V E V E D A L T S S A V K  
 1210 1230 1250

Fig. 10 / continuing 1

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AGGAGAAAGCTGGTGCCTTTACCCCGCACGGTGTCCCGGCTGCCAGGGAGACTG
E K L V R F L P R T V S R L P E E E T E
1270 1290 1310
AGAGTTGGATCAAATGGCTAAAGAAATTCTGAATGTTCTCACCTATTAAACAGTTATTA
S W I K W L K E I L E C S H L L T V I K
1330 1350 1370
AAATGGAAGAAGCTGGGATGAAATTGTGAGCAATGCCATCTCCTACGCTCTACAAAG
M E E A G D E I V S N A I S Y A L Y K A
1390 1410 1430
CCTTCAGCACCAAGTGAGCAAGACAAGGATAACTGGAATGGCAGCTGAAGCTCTGCTGG
F S T S E Q D K D N W N G Q L K L L E
1450 1470 1490
AGTGGAACCGACTGGACCTAGCAATGATGAGATTTCACCAATGACGGCCATGGAGA
W N Q L D L A N D E I F T N D R R W E K
1510 1530 1550
AGAGCAACCCGAGGCTCAGAGACACAATAATCCAGGTACATGGCTGGAAATGGTAGAA
S K P R L R D T I I Q V T W L E N G R I
1570 1590 1610
TCAAGGTTGAGAGCAAAGATGTGACTGACGGCAAAGCCTCTCTCATATGCTGGTGGTTC
K V E S K D V T D G K A S S H M L V V L
1630 1650 1670
TCAAGTCTGCTGACCTTCAAGAAGTCATGTTACGGCTCTCATAAAGGACAGACCAAGT
K S A D L Q E V M F T A L I K D R P K F
1690 1710 1730
TTGTCGCCTCTTCTGGAGAATGGCTGAACTACGGAAAGTTCTCACCCATGATGTC
V R L F L E N G L N L R K F L T H D V L
1750 1770 1790
TCACTGAACTCTTCTCCAACCACCTTCAGCACGCTTGTACCGGAATCTGCAGATGCCA
T E L F S N H F S T L V Y R N L Q I A K
1810 1830 1850
AGAATTCCCTATAATGATGCCCTCCTCACGTTGTCGGAAACTGGTGCAGACTCCGAA
N S Y N D A L L T F V W K L V A N F R R
1870 1890 1910
GAGGCTTCCGAAGGAAGACAGAAATGCCGGACGAGATGGACATAGAACCTCCACGACG
G F R K E D R N G R D E M D I E L H D V
1930 1950 1970
TGTCTCCTTACTCGGCACCCCTGCAAGCTCTTCATCTGGCCATTCTCAGAATA
S P I T R H P L Q A L F I W A I L Q N K
1990 2010 2030
AGAAGGAACTCTCCAAAGTCATTGGGAGCAGACCGAGGGCTGCACTCTGGCAGCCCTGG
K E L S K V I W E Q T R G C T L A A L G
2050 2070 2090
GAGCCAGCAAGCTCTGAAGACTCTGCCAAAGTGAAGAACGACATCAATGCTGGGG
A S K L L K T L A K V K N D I N A A G E
2110 2130 2150
AGTCCGAGGAGCTGGCTAATGAGTACGAGACCCGGCTGTTGGTGAATGCCACAGTGTGGA
S E E L A N E Y E T R A V G E S T V W N
2170 2190 2210
ATGCTGTGGTGGCGCGGATCTGCCATGTGGCACAGACATTGCCAGCGGCACTCATAGAC
A V V G A D L P C G T D I A S G T H R P
2230 2250 2270
CAGATGGTGGAGAGCTGTTCACTGAGTGTACAGCAGCGATGAAGACTTGGCAGAACAGC
D G G E L F T E C Y S S D E D L A E Q L
2290 2310 2330
TGCTGGCTATTCCGTGAAGCTTGGGGTGGAAAGCAACTGTCGGAGCTGGCGGTGGAGG
L V Y S C E A W G G S N C L E L A V E A
2350 2370 2390
CCACAGACCAAGCAATTCACTGCCAGCCCTGGGTCCAGAATTTCATTAAGCAATGGT
T D Q H F I A Q P G V Q N F L S K Q W Y
2410 2430 2450
ATGGAGAGATTCCCGAGACACCAAGAACACTGGAAGATTATCCTGTGTCTGTTATTATAC
G E I S R D T K N W K I I L C L F I I P

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Fig. 10 / continuation 2

2470	2490	2510
CCTTGGTGGCTGGCTTGATCATTTAGGAAGAACCTGTCGACAAGCACAAGAAGC		
L V G C G F V S E R K K P V D K H K K L		
2530	2550	2570
TGCTTTGGTACTATGGCGTCTTCACCTCCCCCTTCGTGGCTTCCTGGATGTGG		
L W Y Y V A F F T S P F V V F S W N V V		
2590	2610	2630
TCTTCTACATCGCCCTCCTCTGCTGTTGCCTACGTGCTGCTCATGGATTCCATTGG		
F Y I A F L L F A Y V L L M D F H S V		
2650	2670	2690
TGCCACACCCCCCCCAGCTGGTCTGTACTCGCTGGCTTGTCTGTGATGAAG		
P H P P E L V L Y S L V F V L F C D E V		
2710	2730	2750
TGAGACAGGGCCGGCCGGCTGCTCCAGTGCAGGGGCCCCAAGCCCCACGCCACCCGGA		
R Q G R P A A P S A G P A K P T P T R N		
2770	2790	2810
ACTCCATCTGGCCCGCAAGCTCCACACGCAGCCCCGGTCCGGCTCACGCCACTCCTCC		
S I W P A S S T R S P G S R S R H S F H		
2830	2850	2870
ACACTTCCCTGCAAGCTGAGGGTGCCTGGCTCTGGCCTTGCCAGCCCCAGAAAGGGGTGGA		
T S L Q A E G A S S G L G Q P R K G W T		
2890	2910	2930
CATTAAAAATCTGGAAATGGTTGATATTCCAAGCTGCTGATGTCCTCTGTCCCTT		
F K N L E M V D I S K L L M S L S V P F		
2950	2970	2990
TCTGTACGCAGTGGTACGTAATGGGTGAATTATTTACTGACCTGTGGATGTGATGG		
C T Q W Y V N G V N Y F T D L W N V M D		
3010	3030	3050
ACACGCTGGGGCTTTTTACTTCATAGCAGGAATTGTATTCGGCAAGGGATCCTTAGGC		
T L G L F Y F I A G I V F R Q G I L R Q		
3070	3090	3110
AGAATGAGCAGCGCTGGAGGTGGATATTCCGTCGGTACAGAGCCCTACCTGGCCA		
N E Q R W R W I F R S V I Y E P Y L A M		
3130	3150	3170
TGTTCGGCCAGGTGCCAGTGACGTGGATGGTACCGTATGACTTGGCCACTGCACCT		
F G Q V P S D V D G T T Y D F A H C T F		
3190	3210	3230
TCACTGGGAATGAGTCCAAGCCACTGTGTGGAGCTGGATGAGCACACCTGGCCCGT		
T G N E S K P L C V E L D E H N L P R F		
3250	3270	3290
TCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTGC		
P E W I T I P L V C I Y M L S T N I L L		
3310	3330	3350
TGGTCAACCTGCTGGTCGCCATGTTGGCTACAOGGTGGCACCGTCCAGGAGAACATG		
V N L L V A M F G Y T V G T V Q E N N D		
3370	3390	3410
ACCAGGTCTGGAAGTTCCAGAGGTACTTCTGGTCAGGAGTACTGCAGCCCTCAATA		
Q V W K F Q R Y F L V Q E Y C S R L N I		
3430	3450	3470
TCCCCCTCCCTCATCGTCTCGCTTACCTCTACATGGTGGTGAAGAAGTGCTCAACT		
P F P F I V F A Y F Y M V V K K C F K C		
3490	3510	3530
GTTGCTGCAAGGAGAAAAACATGGAGTCTCTGCTGCTGTGAGTGGTTATCCATGTGT		
C C K E K N M E S S V C C E W F I H V Y		
3550	3570	3590
ACTTGGGATCAGAAGCAGCGATTAAATTCAAGGAAGGATGCCATCCAGTGATTGGAA		
L G S E A A I N F R E G C L H P V I G S		
3610	3630	3650
GCTGGACCCCAGGCTGGCTGGCTGGACATCCACACGGATTCTCACATGCAGTGGCCGGCT		
W T P G W L V W T S T R I L T C S A G W		
3670	3690	3710
GGCCAGCAGCAGGGAGTCTCACTGTCAACACACATAGCAGCTGGGTTCTGCAAAAAGCA		

Fig. 10 / continued on 3

P	A	A	G	S	L	S	V	T	T	H	S	S	W	V	P	A	K	S	S
3730								3750							3770				
GCAAGTCACAGGCCACCCAGACAGAACGGTAGAGAACATGTGACTCTGCTTCTGGGGGG																			
K	S	Q	A	H	P	D	R	T	G	R	E	C	D	S	A	S	G	W	E
3790								3810							3830				
AAGGACAGCCCTGCCGGTGGGTGGAAGAACATCCGTGGCCCTGTTGGCCATCGTGGCCCTG																			
G	Q	P	A	R	W	V	E	E	S	V	A	L	F	G	H	R	G	P	V
3850								3870							3890				
TTTGGCCACCTACCACTCTAGGCATCACTGAGCTGAATGCGCCGGTCCTCTGA																			
W	P	P	T	T	L	G	I	T	E	L	N	A	P	V	L	*			

MVGGCRWTEDVEPAEVKEKMSFRAARLSMRNRRNDTLDSTRTLYSSASRSTDLSYSESASFYAAFRTOTCPIMASWDLVNFIQANF  
 KKRCVFFTKDSKATENVCKCGYAQSQHMEGTQINQSEKWNKYKHTKEFPTDAFGDIQFETLGKKGYIRLSCDTEAEILYELLTQ  
 HWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIVAGIAAWGMVS  
 NRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNNTHLLLVDNGCHGHPTVEAKLRNQLEKYISERTIQDSNYGGKIPIVCFAQG  
 GGKETLKANTSINKIIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLPRTVSRLPEEETESWIKWLKEILECSHLLTV  
 IKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNGQQLKLLLEWNQLDLANDEIFTNDRRWEKSKPRLRDTIIQVTWLENGRIKVES  
 KDVTDGKASSHMLVVLKSADLQEVMTALIKDRPKFVRLFLENGLNLRKFLTHDVLTLEFSNHFSTLVYRNLLQIAKNSYNDALLTF  
 VVKKLVANFRRGFRKEDRNGRDEMEDIHLHDVSPITRPLQALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVNDIN  
 AAGESEELANEYETRAVGESTVWNNAVVGADLPCGTIASGTHRPDGGEFTECYSSDEDLAEQLLVYSCAEWGGSNCLELAVEATD  
 QHFIAQPGVQNFLSKQWYGEISRDTKNWIIILCLFIIPLVCGFVFSFRKKPVDKKKLWYYVAFFTSPFVVFSWNVVFYIAFLLL  
 FAYVLLMDFHSVPHPPPELVLYSLVFLFCDEVQRGRPAAPSAGPAKPTPTRNSIWPASSTRSPGSRSRHSFHTSLQAEGASSGLQ  
 PRKGWTFKNLEMVDISKLLMSLSVPFCTQWYVNGVNYFTDLWNVMDTLLGFYFIAGIVFRQGILRQNEQRWRWIFRSVIYEPYLM  
 FGQVPSDVDTTYDFAHCTFTGNESKPLCVELDEHNLPRFPEWITIPLVCIYMLSTNILLVNLVAMFGYTVGTVQENNDQVWKFQ  
 RYFLVQEYCSRLNIPFPFIVEFAYFYMVKKCFKCCCKEKNMESSVCCEWFIIHVYLGSEAAINFREGCLHPVIGSWTPGWLWVTSTR  
 ILTCAGWPAAGSLSVTHSSWVPAKSSKSQAHPDRTGRECDASGWEGQPARWVEESVALFGHRGPVWPPTTLGITELNAPVL

B.

		Q L																	
2290		2310																	
TGCTGGTCTATTCCTGTGAAGCTTGGGGTGGAAAGCAACTGTCGGAGCTGGCGGTGGAGG																			
L	V	Y	S	C	E	A	W	G	G	S	N	C	L	E	L	A	V	E	A
2350								2370							2390				
CCACAGACCAGCATTTCATCGCCAGCCTGGGTCCAGAATTTCTTCTAAGCAATGGT																			
T	D	Q	H	F	I	A	Q	P	G	V	Q	N	F	L	S	K	Q	W	Y
2410								2430							2450				
ATGGAGAGATTTCCCGAGACACCAAGAACGAACTGGAAGATTATCCTGTGTCTGTTATTATAC																			
G	E	I	S	R	D	T	K	N	W	K	I	I	L	C	L	F	I	I	P
2470								2490							2510				
CCTTGGTGGCTGTGGCTTGTATCTTAGGAAGAAACCTGTCGACAAGCACAAGAACG																			
L	V	G	C	G	F	V	S	F	R	K	K	P	V	D	K				

Figure 11:

a.) Trp10b cDNA and derived amino acid sequence

10	30	50
ATGAAATCCTCCTTCTGTCCACACCCATCGTGTATCAGGGAGAATGTGTGCAAGTGT		
M K S F L P V H T I V L I R E N V C K C		
70	90	110
GGCTATGCCAGAGCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGGAGAAATGGAAC		
G Y A Q S Q H M E G T Q I N Q S E K W N		
130	150	170
TACAAGAACACACCAAGGAATTCTACCGACGCCCTGGGGATATTCAAGTTGAGACA		
Y K K H T K E F P T D A F G D I Q F E T		
190	210	230
CTGGGAAAGGAAGGTATACGTCTGCTCGACACGGACGGAAATCCTTAC		
L G K K G K Y I R L S C D T D A E I L Y		
250	270	290
GAGCTGCTGACCCAGCACTGGCACCTGAAAACACCCAACCTGGTCATTCTGTGACCGGG		
E L L T Q H W H L K T P N L V I S V T G		
310	330	350
GGCGCCAAGAACCTCGCCCTGAAGCCGCGCATCGCAGATCTCAGCCGGCTCATCTAC		
G A K N F A L K P R M R K I F S R L I Y		
370	390	410
ATCGCGCAGTCCAAGGGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAG		
I A Q S K G A W I L T G G T H Y G L M K		
430	450	470
TACATGGGGAGGTGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTG		
Y I G E V V R D N T I S R S S E E N I V		
490	510	530
GCCATTGGCATAGCAGCTGGGCATGGCTCTCAACCGGGACACCCCTCATCAGGAATTGC		
A I G I A A W G M V S N R D T L I R N C		
550	570	590
GATGCTGAGGGCTATTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTG		
D A E G Y F L A Q Y L M D D F T R D P L		
610	630	650
TATATCTGGACAACAACCACACATTGCTGCTCGTGGACAATGGCTGTCATGGACAT		
Y I L D N N H T H L L L V D N G C H G H		
670	690	710
CCCACTGTCGAAGCAAAGCTCCGGAATCAGCTAGAGAGAAGTATATCTCTGAGCGCACTATT		
P T V E A K L R N Q L E K Y I S E R T I		
730	750	770
CAAGATTCCAACATGGTGGCAAGATCCCCATTGTGTGTTTGCCCAAGGAGGTGGAAAA		
Q D S N Y G G K I P I V C F A Q G G G K		
790	810	830
GAGACTTTGAAAGCCATCAATACTCCATCAAAATAAAATTCTGTGTTGGGGAA		
E T L K A I N T S I K N K I P C V V V E		
850	870	890
GGCTGGGCCAGATCGCTGATGTGATCGTAGCCTGGGGAGGTGGAGGATGCCCTGACA		
G S G Q I A D V I A S L V E V E D A L T		
910	930	950
TCTTCTGCCGTCAAGGAGAAGCTGGTGCCTTTACCCCGCACGGTGTCCCGCTGCCT		
S S A V K E K L V R F L P R T V S R L P		
970	990	1010
GAGGAGGAGACTGAGAGTTGGATCAAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTA		
E E E T E S W I K W L K E I L E C S H L		
1030	1050	1070
TTAACAGTTATTAATGGAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTAC		
L T V I K M E E A G D E I V S N A I S Y		
1090	1110	1130
GCTCTATACAAAGCCTTCAGCACCAAGTGGAGCAAGACAAGGATACTGGAATGGCAGCTG		
A L Y K A F S T S E Q D K D N W N G Q L		

Fig. 11 (Continuation)

2410	2430	2450
AGAAACTTAGGACCCAAAGATTATAATGCTGCAGAGGATGCTGATCGATGTGTTCTTCTTC		
R N L G P K I I M L Q R M L I D V F F F		
2470	2490	2510
CTGTTCCCTTTGCGGTGTGGATGGTGGCCTTGGCGTGGCCAGGCAAGGGATCCTTAGG		
L F L F A V W M V A F G V A R Q G I L R		
2530	2550	2570
CAGAACGAGCAGCGCTGGAGGTGGATATTCCGTTGGTCATCTACAGGCCCTACCTGGCC		
Q N E Q R W R W I F R S V I Y E P Y L A		
2590	2610	2630
ATGTTGGCCAGGTGCCAGTGACGTGGATGGTACCACTACGTATGACTTTGCCACTGCACC		
M F G Q V P S D V D G T T Y D F A H C T		
2650	2670	2690
TTCACTGGGAATGAGTCCAAGCCACTGTGTGGAGCTGGATGAGCACAAACCTGCCCGG		
F T G N E S K P L C V E L D E H N L P R		
2710	2730	2750
TTCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTG		
F P E W I T I P L V C I Y M L S T N I L		
2770	2790	2810
CTGGTCAACCTGCTGGTCGCCATGTTGGCTACACGGTGGCACCGTCCAGGAGAACAT		
L V N L L V A M F G Y T V G T V Q E N N		
2830	2850	2870
GACCAGGTCTGGAAGTCCAGAGGTACTTCTGGTGCAGGAGTACTGCAGGCCCTCAAT		
D Q V W K F Q R Y F L V Q E Y C S R L N		
2890	2910	2930
ATCCCCCTCCCTTCATCGTCTCGCTTACTTCTACATGGTGGTAAGAAAGTGCTCAAG		
I P F P F I V F A Y F Y M V V K K C F K		
2950	2970	2990
TGTTGCTGCAAGGAGAAAAACATGGAGTCTCTGTCTGCTGTTCAAAATGAAGACAAAT		
C C C K E K N M E S S V C C F K N E D N		
3010	3030	3050
GAGACTCTGGCATGGAGGGTGTCAAGGAAAACACTACCTTGCAAGATCAACACAAAAA		
E T L A W E G V M K E N Y L V K I N T K		
3070	3090	3110
GCCAAACGACACCTCAGAGGAATGAGGCATCGATTTAGACAACACTGGATAACAAAGCTTAAT		
A N D T S E E M R H R F R Q L D T K L N		
3130	3150	
GATCTCAAGGGTCTACTGAAAGAGATTGCTAATAAAATCAAATAG		
D L K G L L K E I A N K I K *		

## b.) Trp10 protein:

MKSFLPVHTIVLIRENVCKCGYAQSQHMEGTQINQSEKWNYYKKHTKEFPTDAFGDIQFETLGKKKYIRLSCDTEAILEY  
 ELLTQHWHLKTPNLVISVTGGAKNFKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIV  
 AIGIAAWGMVSNRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNHHTLLLVDNGCHGHTVEAKLRNQLEKYISERTI  
 QDSNYGGKIPIVCFAQGGGKETLKAIANTSINKIIPCVVVVEGSGQIADVIASLVEVEDALTSAVKEKLVRFLPRTVSRLP  
 EEEETESWIKWLKEILECSHLLTVIKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNGQLKLLLEWNQLDLANDEIFTND  
 RRWESADLQEVMFTALIKDRPKFVRLFLEGLNLRKFLTHDVLTTELFSNHFSTLVYRNLIQAKNSYNDALLTFVWKLVAN  
 FRRGFRKEDRNGRDEMDIELHDVSPITRHPLQALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDINA  
 AGESEELANEYETRAVELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEATDQHFIQPGVQNFLSKQWYGEISRDT  
 KNWKIIILCLFIIPLVCGFVFSRKPKVDHKKLLWYYVAFFTSPFVVFWSWNVVFYIAFLLLFAYVLLMDFHSPHPPELV  
 LYSLVFVLFCDERQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIFTLRLIHIFTVS  
 RNLGPKIIIMLQRLMIDVFFFLFLFAVWMVAFGVARQGILRQNEQRWRWIFRSVIYEPYLAMFGQVPSDVGTTYDFAHCT  
 FTGNESKPLCVELDEHNLPRFPEWITIPLVCYIYLSTNILLVNLLVAMFGYTVGTVQENNDQVWKFQRYFLVQEYCSRIN  
 IPFPFIVFAYFYMVVKCFKCCKEKNMESSVCCFKNEDNETLAWEGVMKENYLVKINTKANDTSEEMRHRFRQLDTKLN  
 DLKGLLKEIANKIK

Figs. 12A and 12B

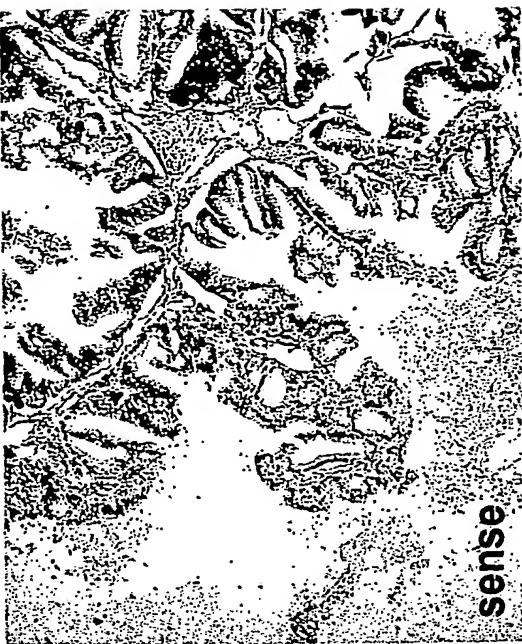
**The Trp8 gene is expressed in endometrial or uterine cancer, but not in normal endometrium**

**Endometrial cancer:**

**A**



**B**



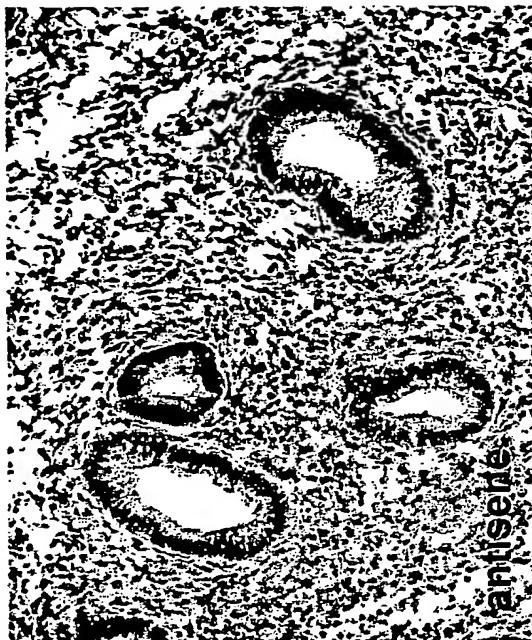
Figs. 12C and 12D



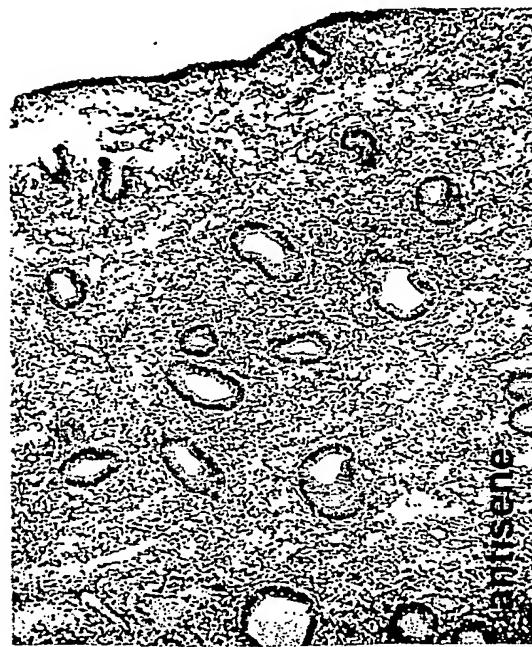
Figs. 12E and 12F

**Endometrium:**

**F**



**E**



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Fig. 13

## Expression of human Trp 9 and Trp 10

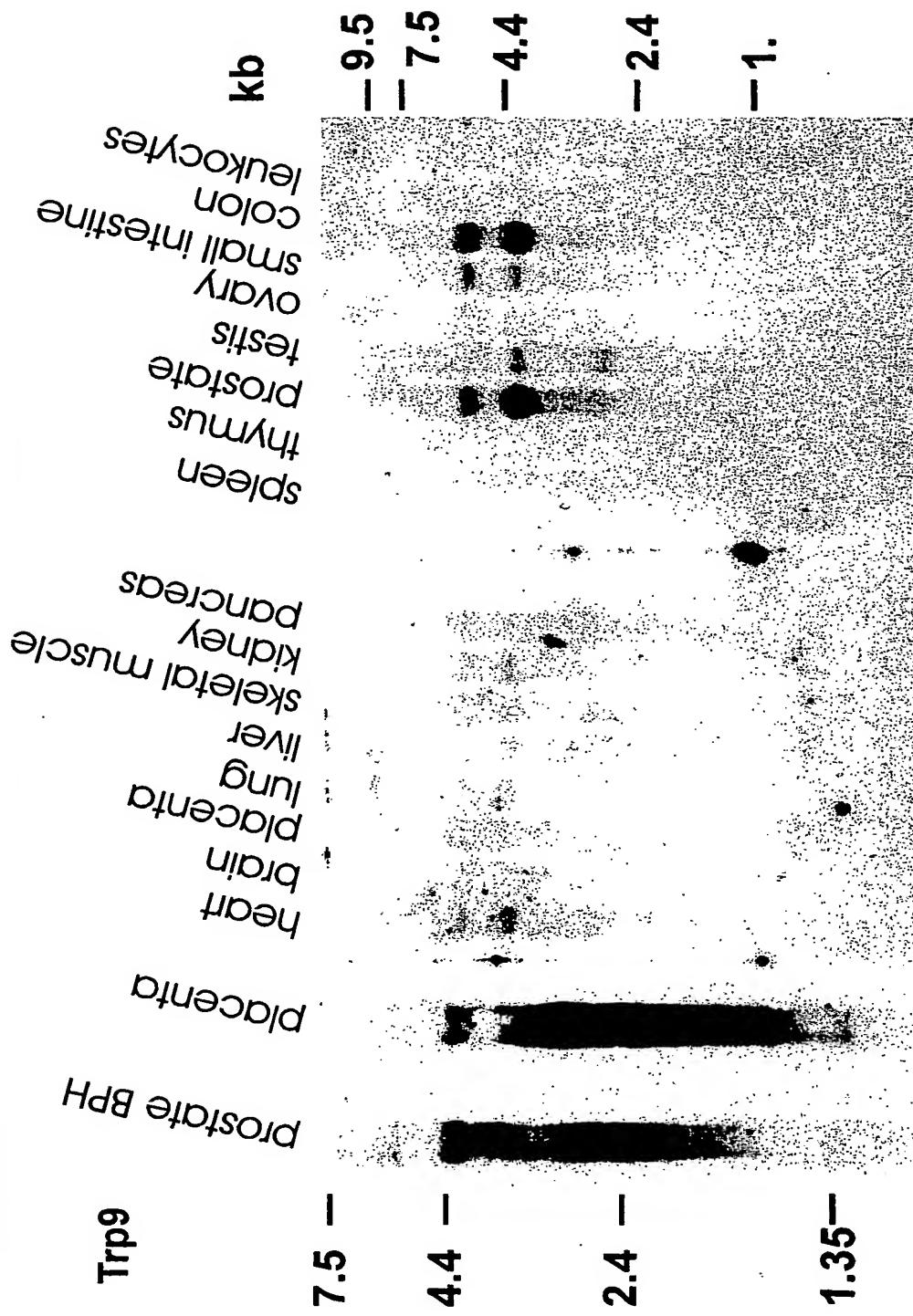
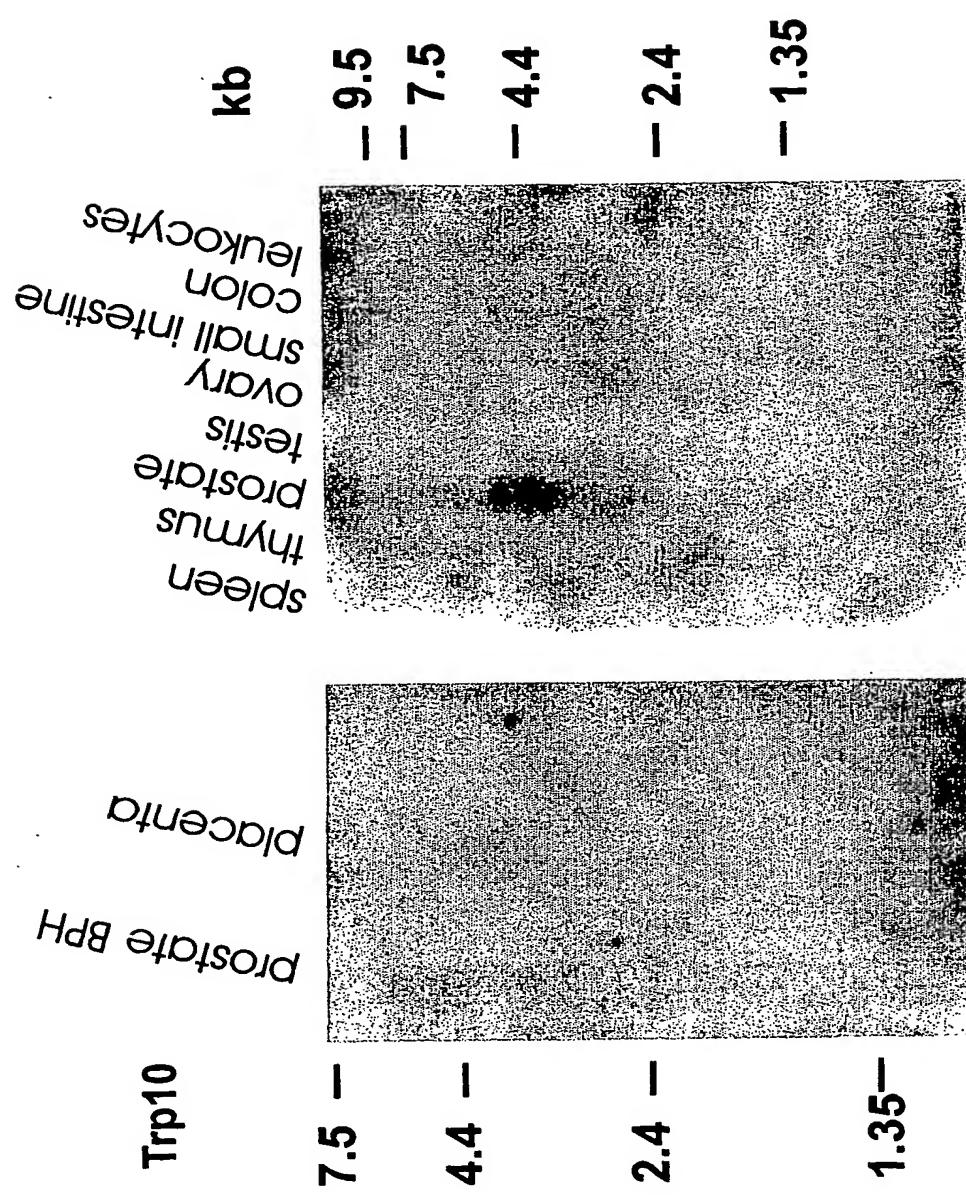
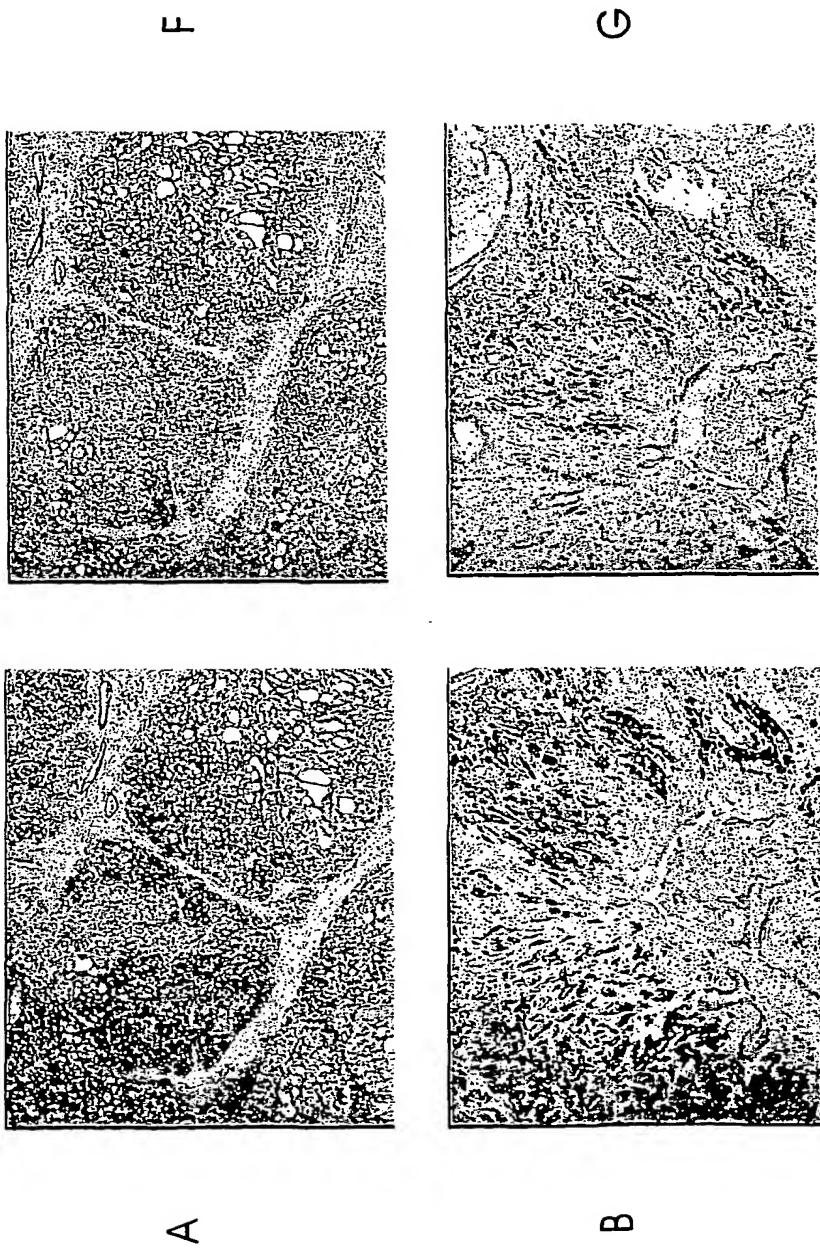


Fig. 13 / Continuation 1



Figs. 14A, 14B, 14F and 14G

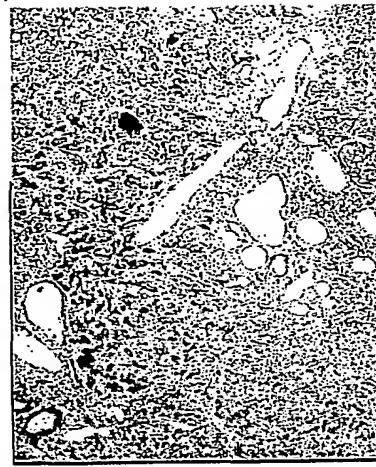
Expression of Trp10 transcripts and Trp10-antisense transcripts  
in human prostate cancer and in malignant melanoma



H



J



C



D



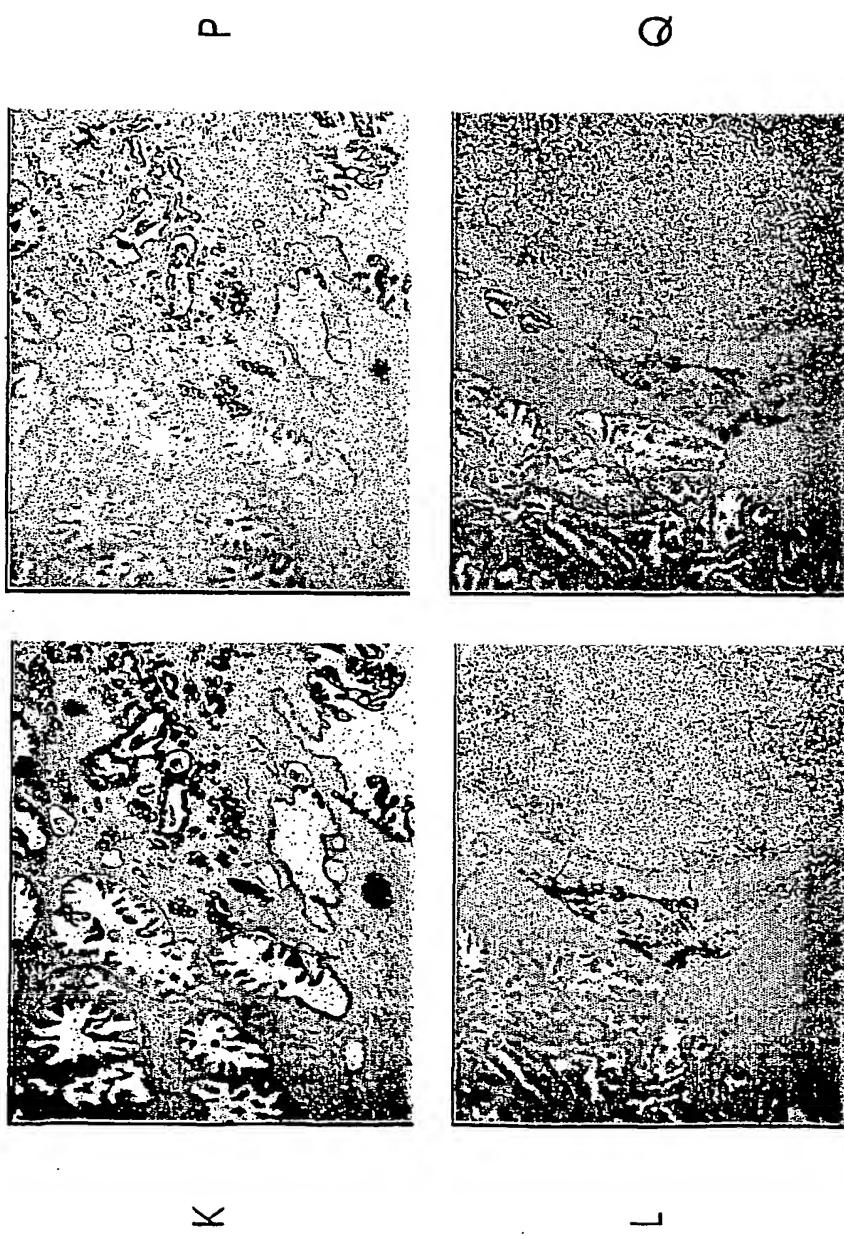
E



Figs. 14C, 14D, 14E, 14H, 14I and 14J

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Figs. 14K, 14L, 14P and 14Q



K

L

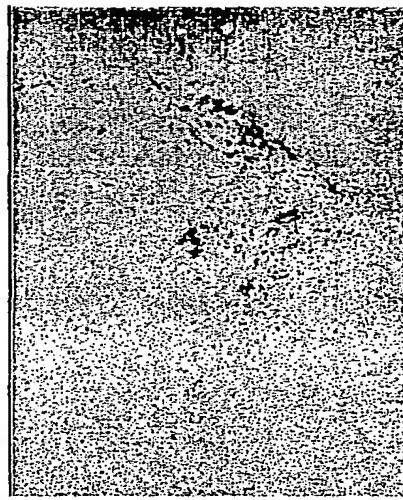
R



S



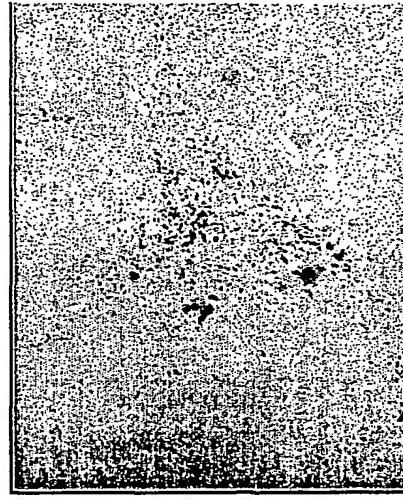
T



M



N



O

Figs. 14M, 14N, 14O, 14R, 14S and 14T



(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
7 February 2002 (07.02.2002)

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(71) Applicant and

(72) Inventor: WISSENBACH, Ulrich [DE/DE]; Institut für Pharmakologie und Toxikologie der Uni, versität des Saarlandes, 66421 Homburg (DE).

(74) Agent: HUBER, Bernard; Huber & Schüssler, Truderinger Str. 246, 81825 München (DE).

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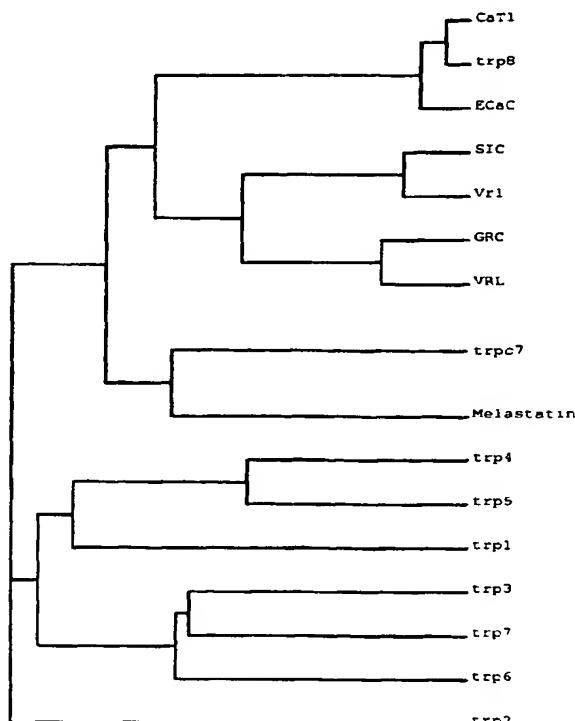
(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## Published:

— with international search report

[Continued on next page]

(54) Title: TRP8, TRP9 AND TRP10, MARKERS FOR CANCER



(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

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**(88) Date of publication of the international search report:**

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

**(15) Information about Correction:****Previous Correction:**

see PCT Gazette No. 38/2002 of 19 September 2002, Section II

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 01/08309

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7	C12N15/12	C12N15/11	C12N9/00	C07K14/47	C12Q1/68
	G01N33/577	A61K31/713		C07K14/705	

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, SEQUENCE SEARCH, WPI Data, PAJ

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 09166 A (SHAPERO MICHAEL H ;DENDREON CORP (US); LAUS REINER (US); TSAVALER) 25 February 1999 (1999-02-25) see SEQID14 + 15, pages 2,3, 28,29, Example 4 table 3 ---	1-10, 12-17, 23,29-31
X	WO 00 40614 A (BETH ISRAEL HOSPITAL ;SCHARENBERG ANDREW M (US)) 13 July 2000 (2000-07-13) see seqid31 + 32, page 11, first paragraph, page 44, lines 13-15 --- -/-	1-10,12, 31

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search

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Date of mailing of the international search report

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Holtorf, S

## INTERNATIONAL SEARCH REPORT

National Application No

PCT/EP 01/08309

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MULLER D ET AL: "Molecular cloning, tissue distribution, and chromosomal mapping of the human epithelial Ca <sup>2+</sup> channel (ECAC1)." GENOMICS, vol. 67, no. 1, 1 July 2000 (2000-07-01), pages 48-53, XP002222953 ISSN: 0888-7543 the whole document ---	1
X	WO 98 15657 A (ABBOTT LAB) 16 April 1998 (1998-04-16) the whole document ---	1-12, 29-31
X	WO 98 37093 A (CORIXA CORP) 27 August 1998 (1998-08-27) the whole document ---	1-12, 29-31
A	TSAVALER LARISA ET AL: "TRP-P8, a novel prostate-specific gene, is upregulated in prostate cancer and other malignancies and shares high homology with TRP calcium channel proteins." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, no. 41, March 2000 (2000-03), page 694 XP008011242 91st Annual Meeting of the American Association for Cancer Research.; San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X the whole document ---	
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P,X	WO 01 14423 A (SMITHKLINE BEECHAM PLC) 1 March 2001 (2001-03-01) see SEQid1 + 2; see example 1 ---	1-9,31
		-/-

## INTERNATIONAL SEARCH REPORT

National Application No  
PCT/EP 01/08309

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		Relevant to claim No.
Category *	Citation of document, with indication, where appropriate, of the relevant passages	
P,X	WISSENBACH ULRICH ET AL: "Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 22, 1 June 2001 (2001-06-01), pages 19461-19468, XP002222954 ISSN: 0021-9258 the whole document	1-9,13, 14, 16-19, 21-23,29
P,X	WO 01 04303 A (HEDIGER MATTHIAS A) 18 January 2001 (2001-01-18) see SEQID1 + 2 the whole document	1-5
P,X	WO 01 42467 A (MILLENNIUM PREDICTIVE MEDICINE) 14 June 2001 (2001-06-14) see SEQID 4615	1
E	WO 01 51633 A (FANGER GARY RICHARD ;HARLOCKER SUSAN L (US); MEAGHER MADELEINE JOY) 19 July 2001 (2001-07-19) see SEQID764, example 3, claims	1
E	WO 02 14361 A (AGENSYS INC) 21 February 2002 (2002-02-21) see SEQID1479, examples 1-4 the whole document	1-10, 13-23
E	WO 02 00722 A (SILOS SANTIAGO INMACULADA ;CURTIS RORY A J (US); MILLENNIUM PHARM) 3 January 2002 (2002-01-03) see SEQID4	1-5
E	WO 01 68857 A (CURTIS RORY A J ;COOK WILLIAM JAMES (US); MILLENNIUM PHARM INC (US) 20 September 2001 (2001-09-20) see SEQID1, examples	1-5
E	WO 01 53348 A (SQUIBB BRISTOL MYERS CO ;GAUGHAN GLEN T (US); RAMANATHAN CHANDRA S) 26 July 2001 (2001-07-26) see SEQID5 the whole document	1
E	WO 01 62794 A (LORA JOSE M ;CURTIS RORY A J (US); GLUCKSMANN MARIA ALEXANDRA (US)) 30 August 2001 (2001-08-30) the whole document	1-9
E	WO 02 30268 A (EOS BIOTECHNOLOGY INC) 18 April 2002 (2002-04-18) see SEQID53	1,6
		-/-

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 01/08309

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	BOEDDING MATTHIAS ET AL: "The recombinant human TRPV6 channel functions as Ca <sub>2+</sub> sensor in human embryonic kidney and rat basophilic leukemia cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 39, 27 September 2002 (2002-09-27), pages 36656-36664, XP002222955 September 27, 2002 ISSN: 0021-9258 the whole document -----	

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 01/08309

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 24-28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: 12 partially because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12, 29-31 partially, 13-28 completely

Isolated nucleic acid molecules encoding human prostate carcinom associated proteins as characterized by SEQIDs 5,45,11,3 and SEQIDs 6,46,12,4, respectively; the recombinant expression of the same in host cells; the isolated proteins as characterized by SEQIDs 6,46,12,4; antisense RNA sequence and ribozyme complementary to said nucleic acid molecules; inhibitor that can suppress the activity of said prostate carcinom associated proteins; method for diagnosing a prostate carcinoma by contacting a sample with a nucleic acid, an antibody or other reagent that reacts with the mRNA of SEQIDs 5,45,11,3; method for diagnosing endometrial cancer by contacting a target sample with a nucleic acid, an antibody or other reagent that reacts with the mRNA of SEQIDs 5,45,11,3; method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate comprising contacting a target sample with a reagent which detects antisense RNA of SEQIDs 11 and 3; method for preventing prostate tumour, endometrial cancer, chorion carcinoma or cancer of the lung comprising administering an inhibiting reagent of human prostate carcinom associated proteins; diagnostic kit containing an antibody; method for identifying an agonist or an antagonist of human prostate carcinom associated proteins.

2. Claims: 1-12, 29-31 partially

Isolated nucleic acid molecule encoding human prostate carcinom associated protein as characterized by SEQIDs 7 and SEQIDs 8, respectively; the recombinant expression of the same in host cells; the isolated protein as characterized by SEQIDs 8; antisense RNA sequence and ribozyme complementary to said nucleic acid molecule; inhibitor that can suppress the activity of said prostate carcinom associated protein; diagnostic kit containing an antibody; method for identifying an agonist or an antagonist of human prostate carcinom associated proteins.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 12 partially

Present claim 12 relates to an inhibitor which is defined by reference to a desirable characteristic or property, namely suppressing the activity of the protein of claim 6.

The claims cover all inhibitors having this characteristic or property, whereas the application provides only support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for a limited number of such inhibitors.

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the inhibitors by reference to a result to be achieved.

Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claim 12 which appear to be clear, supported and disclosed, namely those parts relating to the Trp8/10 corresponding antibody, Trp8/10 corresponding antisense construct, a Trp8/10 corresponding ribozyme.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/EP 01/08309

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 9909166	A	25-02-1999	US	6194152 B1		27-02-2001
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			PL	335348 A1		25-04-2000
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